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THE RESPONSES OF THE BLOOD VESSELS OF THE MUSCLES WITH SPECIAL REFERENCE TO THEIR CENTRAL CONTROL

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Although some work has been done on the control of the blood vessels of the muscles, it is very largely prior to 1920 when our knowledge of the control of the circulation was much less. Now, however, that it is realized that the vessels within the muscles are capable of taking up a very large proportion of the blood in the body, the importance of the subject becomes evident. The present experiments were carried out to discover to what extent these vessels are controlled centrally and how this control affects their responses.

METHODS

The experiments were carried out in cats anaesthetized with chloralose and rested until the preliminary stimulation produced by the initial anaesthesia and operative procedures had passed off. This stage is usually reached in about an hour and is indicated by the pupil returning to its normal state of constriction and is considered important in securing the best normal responses. The vessels were studied by enclosing a skinned hind-limb in a plethysmograph in the usual way, the foot being tied off. In many cases comparisons were similarly made on the other normal limb. In all cases a rise in kymograph records from limbs indicates increase in volume of the limb. In all experiments a recorder, already described by McDowall (1928*b*), was used with the important addition of a shaking device which not only prevents effects of surface action on the bell of the recorder but at the same time makes the records more easily photographed.

RESULTS

The normal tonic activity of the vasoconstrictor centre and the effects of denervation

The vessels of the muscles are apparently in a tonic state under the control of the vasomotor centre through the sympathetic outflow, for the skinned limb volume increases markedly when the abdominal sympathetic chain is cut at the level of the third lumbar vertebra. This may be done without disturbance of the plethysmograph (Fig. 1), but has not hitherto been recorded. In some

cases, the first, second and third lumbar ganglia were removed. It was not possible to make records of denervation by section of the sciatic and anterior crural nerves, but studies after such denervation gave similar results.

Fig. 1. The effect of section of the abdominal sympathetic chain on the volume of a skinned limb (upper trace), rise indicates an increase in volume and blood pressure (lower trace). Time 30 sec.

Increased activity of the constrictor centre

This may be produced in several ways; not all have similar effects on the vessels in the muscles, although all cause a rise of blood pressure. They are, therefore, considered separately.

Haemorrhage. Haemorrhage always produces a diminution in the size of the skinned and unskinned limb even if it is insufficient to lower the arterial blood pressure permanently (Fig. 2). The vascular bed in the muscles, therefore, takes part in the normal compensation for haemorrhage. In the course of these experiments many attempts were made to obtain the sudden dilatation of muscle vessels which has been shown by Barcroft & Edholm (1945) to occur in man when blood is withdrawn by the application of a cuff which obstructs the venous return, but with negative results.

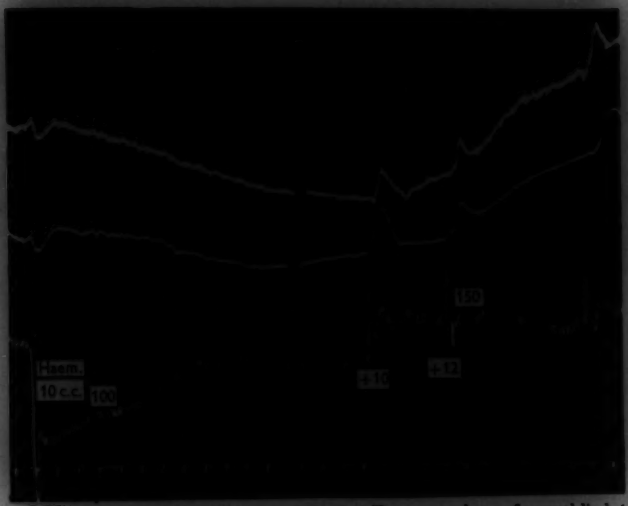


Fig. 2. The effect of the removal of 10 c.c. of blood at Haem. on volume of normal limb (upper trace), skinned limb (middle trace) and blood pressure (lowest trace). At +10, 10 c.c. of warm saline solution was injected intravenously and 12 c.c. at +12. Time 30 sec.

Fig. 3. The effect of carotid occlusion on skinned limb volume (upper trace) and blood pressure (lower trace). *c.c.*, carotids closed; *off*, carotid opened. Time 30 sec.

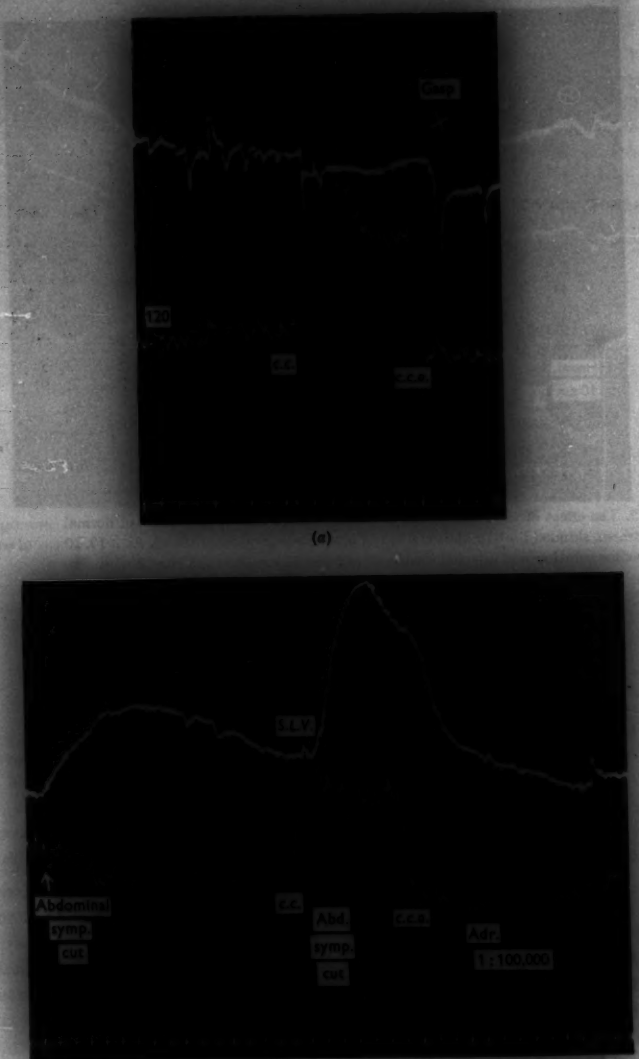


Fig. 4. The effect of carotid occlusion (*c.c.–c.c.c.*) on blood pressure (lower trace) and skinned limb volume (upper trace), (*a*) before and (*b*) after section of the abdominal sympathetic at first arrow. Note the absence of dilatation to adrenaline (1 c.c. of 1:100,000) later.

The carotid sinus reflex. If the blood pressure is recorded from one carotid artery, occlusion of the other brings about the well-known rise of blood pressure due to a reduction of the depressor impulses from the carotid sinus. Painting the sinus with a local anaesthetic such as cocaine, or preferably percaine, which is less toxic, produces a similar rise. The volume of the skinned limb may increase slightly, but it is not usually affected by carotid occlusion (Fig. 3); and this may be followed by a dilatation of the muscle vessels when

Fig. 5. Records of skinned limb volume (above) and blood pressure (below). The carotid was clipped between the arrows at c.c. and adrenaline was injected (0·5 c.c. of 1:10,000) at the arrow *A*.

blood is again allowed into the sinus. In some twenty experiments only one instance of a diminution of volume was recorded. If, however, the lumbar sympathetic chain is cut, the absence of effect may be converted into an increase in volume which is presumably passive (Fig. 4*a*, *b*). A comparison with the effect of adrenaline is very striking (Fig. 5). After section of the vagi, however, application of the carotid clip may lead to a diminution of limb volume. The vagal section leads to a great increase in the blood-pressure

Fig. 6a. The effect of carotid occlusion between arrows on the outflow of blood in drops from the femoral vein in a heparinized cat. Records from above downwards, arterial blood pressure, venous blood pressure, outflow in drops from femoral vein. Time 30 sec.

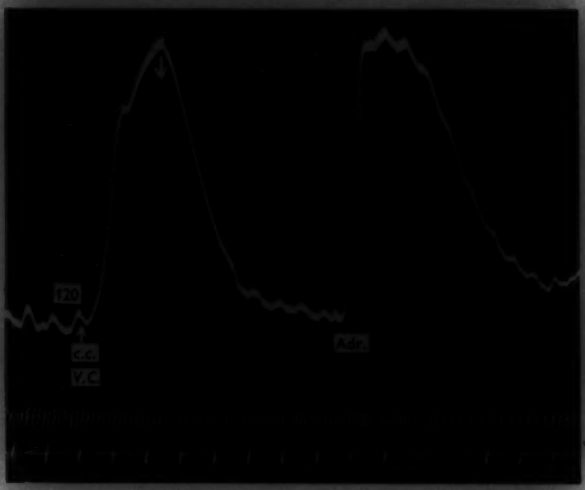


Fig. 6b. The effect of cerebral anaemia at *c.c. VC* and of adrenaline on femoral outflow and blood pressure (above). Time 10 sec.

response to carotid occlusion also presumably because the usual response to the occlusion tends to be antagonized by the cardio-aortic reflex.

The unexpected finding of absence of any immediate change of limb volume associated with the rise of blood pressure when the carotids only are occluded led to the study of the response in other ways: (1) by the innervated perfusion method (McDowall, 1932) in which an innervated skinned limb is separately perfused; and (2) by recording the outflows of blood from the femoral vein.

Fig. 7. The effect of asphyxia (*Asp.*) compared with carotid occlusion (*c.c.*). Note that the asphyxia produces a diminution of the volume of the skinned limb (*S.L.V.*), while carotid occlusion, producing a similar rise of blood pressure (lower record), does not. The normal limb volume, *N.L.V.*, increases in both cases.

Both methods showed that there is almost always a marked constriction of the muscle vessels. The possible significance of these findings is discussed later. It will be noticed that the period of intense vasoconstriction is followed by an increased flow apparently due, like the increased volume in Fig. 3, to the return of blood to the sinus, but partly to a reactive hyperaemia (Fig. 6*a*), for it is present after the sinus has been paralysed by percaine. It was unfortunately not found possible to take simultaneous records of volume and femoral outflow as the skinning of both hind-limbs invariably resulted in shock.

Cerebral anaemia. If a clamp (McDowall, 1930) is placed on the vertebral arteries, complete cerebral anaemia may be produced in the majority of cats (Hill, 1896) by occluding the remaining common carotid artery, the other having already been used for the recording of blood pressure. This procedure produces the largest rise of arterial pressure it is possible to obtain in a cat, sometimes to 400 mm. Hg. Again, as in the case of the carotid sinus reflex, there is usually little change in the skinned limb volume at a time when the normal limb volume has already begun to decrease. Appreciably later, the volume of the skinned limb decreases too. If the response is compared with that to a dose of adrenaline which gives a comparable rise of arterial pressure, the difference is again very marked (Fig. 6*b*).

Asphyxia. Asphyxia leads to a diminution of skinned limb volume. This occurs even if the adrenal glands have been previously tied off. The normal limb, on the other hand, may dilate in spite of the constriction of the underlying muscle vessels (Fig. 7), but constriction may also be seen.

Lactic acid. Intravenous injection of dilute lactic acid solution also causes a constriction of the vessels of the innervater muscles (Fig. 11*b*) (see also p. 11).

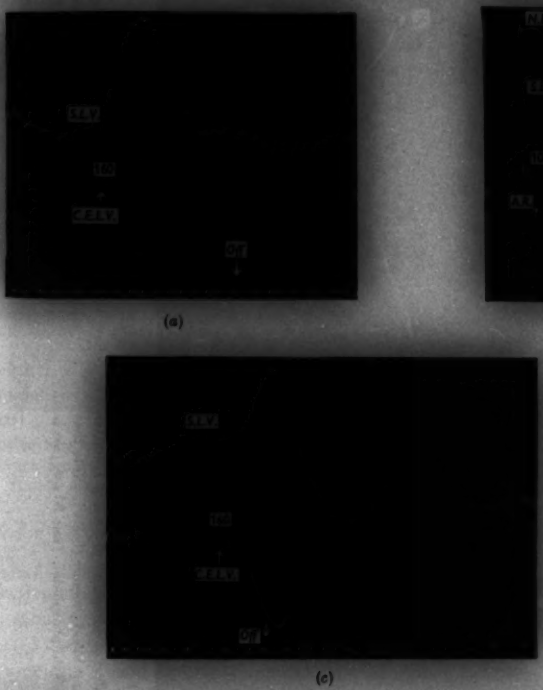
Reduction of the activity of the vaso-constrictor centre by stimulation of the central end of the vagus

In the cat it is well known that it is often difficult to obtain good depressor effects on the blood pressure because a separate depressor nerve does not always exist and because of the admixture of pressor fibres (McDowall, 1924). Even when good effects are obtained the action on the vessels of the limb muscles varies very much. Sometimes, particularly at the beginning of an experiment (Fig. 8*a*), a dilatation of muscle vessels is followed by a constriction which may be due to the stimulation of the pressor fibres or passive, i.e. due to the fall of blood pressure. In other experiments, or later on in the same experiment, a typical dilatation may be seen. Commonly, however, there is no effect whatever, although there is no doubt regarding the freedom of the recording apparatus (Fig. 8*b*). In Fig. 8*c* a compensating constriction of the skin vessels is also well seen. Generally the higher the arterial blood pressure the easier it is to obtain the depressor response, presumably because the tone of the vasoconstrictor centre is high. Raising this tone by haemorrhage may increase the response to depressor stimulation, but admittedly variable results may be obtained in the same animal for no apparent reason. The important point is that the muscle vessels of the cat may participate in the reflex—as pointed out by Jarisch (1925) in the gastrocnemius of the rabbit—but not necessarily so.

Effect of adrenaline

It is well known that adrenaline dilates the vessels of the muscles even in doses insufficient to raise the arterial pressure, or which even produce a fall of

blood pressure (Fig. 9*a*). If the abdominal sympathetic chain is cut, the dilator response to adrenaline is completely but temporarily abolished, presumably because the vessels concerned are already dilated (Hoskins, Gunning & Berry, 1916). This has already been reported by several observers (Hartman & Fraser, 1917), and the subject has been exhaustively discussed by Dale & Richards



(1918) in regard to intact limbs. When the vessels are fully dilated, the injection of a moderate dose of adrenaline may, however, cause vasoconstriction in the vessels of the muscles (Fig. 9*b*). On the other hand, increased activity of the vasoconstrictor centre such as is produced by carotid occlusion reduces, as might be expected, the dilator action of adrenaline (Fig. 10).

Fig. 9. The effect of adrenaline (0·5 c.c. of 1:100,000); (*a*) before, and (*b*) after sympathectomy on skinned limb volume (above) and blood pressure (below). Note that in (*b*) adrenaline causes constriction while histamine dilates (0·1 mg. histamine acid phosphate). Time 30 sec. (see text).

It has been found in the present experiments that the dilator response to adrenaline may return a few hours after section of the sympathetic chain. Other observers, for example, Dale & Richards (1918), found that the response to adrenaline returns after a variable time, but did not suggest any reason for the variation. By the use of choralose anaesthesia it would appear that the capillaries can regain their tone very rapidly or it may never be lost, and the response to histamine may be present before that to adrenaline returns (Fig. 9*b*). The importance of anaesthetics in the disappearance of capillary tone was pointed out by Hemingway & McDowall (1926).

Fig. 10. The effects of carotid occlusion (*c.c.–c.c.o.*) in reducing the effect of adrenaline at each arrow (0·5 c.c. of 1:100,000) on skinned limb (*S.L.V.*). B.P., blood-pressure record. Time 30 sec.

Effect of pH

The return of tone after denervation is very much hastened by intravenous injection of sodium bicarbonate solution which reduces the volume of the limb immediately. Especially after sympathetic section, it can be readily demonstrated that the denervated vessels can be easily constricted by alkali or dilated by amounts of acid *which do not affect the general blood pressure* (Fig. 11*a*). The injection of the acid or alkaline solutions commonly causes a series of steps in the plethysmograph record which do not appear on the blood-pressure record. Even more striking results may be obtained if the reactions of a denervated skinned limb to acid after alkali are compared with those of a normal limb connected with the central nervous system (Fig. 11*b*). The former dilates but the latter constricts, indeed there may be, in this case, sufficient generalized vasoconstriction to raise the arterial pressure, as shown in Fig. 11*b*. This is presumably the result of the effect of acid or of the carbon dioxide released on the vasomotor centre. But the blood-pressure response is very variable and probably depends on the state of the centre itself.

(a)

(b)

Fig. 11. The effects of 2 c.c. doses of saturated sodium bicarbonate and lactic acid (0·5 c.c. of 5% solution) on recently denervated, skinned limb (*S.L.V.*) without change of blood pressure. Time 30 sec. In (*b*) the effects on the injection of the same doses of lactic acid are shown on denervated (lower record), innervated skinned limb volume (upper record) and on blood pressure (B.P.).

Effect of histamine

The normal vaso-dilator response to histamine was first demonstrated by Dale & Richards (1918) and may also be abolished by denervation, but they did not differentiate between blood vessels in muscle and those in skin. They recorded, however, that denervation is rapidly followed by an exaggerated response to the drug. In the present experiments, the dilatation by histamine was often seen immediately after section of the sympathetic chains, especially

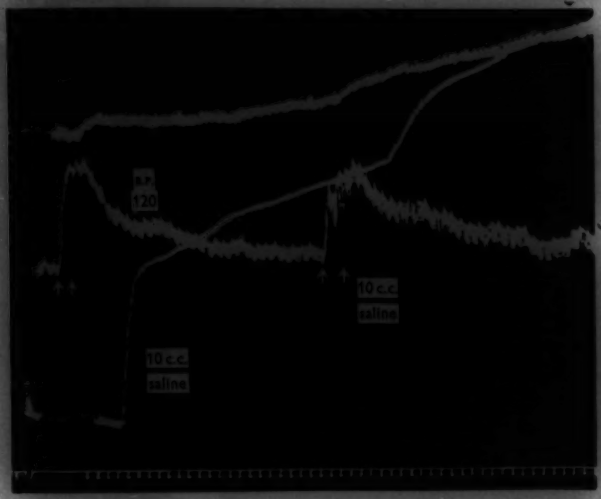


Fig. 12. The effect of the injection of saline solution in the normal (upper trace) and denervated limb (lower trace). Comparisons of limbs (Fig. 2) show that the rapid rise of volume occurs in the muscles. Injections between the arrows. Time 30 sec.

if the capillaries were constricted by the injection of sodium bicarbonate solution, although dilatation with adrenaline might still be absent (Fig. 9*b*). As is seen in this figure, the use of histamine may temporarily reverse the constrictor response to adrenaline which subsequently returns. A potentiating action of histamine on adrenaline dilatation has already been described (McDowall, 1942).

The effects of saline injection and central control

If Ringer's solution is injected intravenously, the increase in muscle volume is very marked (Fig. 2), and this is particularly so if the vessels are denervated (Fig. 12). It is not generally realized that when saline is injected into the

circulation the well-known short-lived effect on the blood pressure is due to the fact that the saline may pass immediately into the vascular bed of the muscles. This occurs so rapidly that it appears unlikely that it has time to leave the

Fig. 13.

Fig. 14.

Fig. 13. The occurrence of rhythmical activity of the muscle vessels (*S.L.V.*) reflected in the blood pressure (B.P.).

Fig. 14. The effect of a fall of arterial pressure produced by stimulation of the peripheral end of the left vagus (between the arrows) on the volume of the skinned (*S.L.V.*) and unskinned (*N.L.V.*) limb. Time 30 sec.

blood vessels as is commonly supposed. Fig. 12 shows the great speed with which this can occur in a denervated limb: a comparison with a normal limb is shown. The recording systems of both plethysmographs were identical, and since skinned and unskinned limbs behave almost identically we may presume

that the increase in volume in a normal limb is chiefly due to the opening up of vessels in muscle. It is unfortunately not possible to compare denervated and innervated skinned limbs in the same animal because of the occurrence of shock.

Rhythmical activity of the vessels

In the course of this investigation two instances were observed when there was apparently rhythmical activity of the vessels without any corresponding change in the blood pressure (Fig. 13). They produce an appearance like Traube-Hering waves.

Response to change in internal blood pressure

In many, but not all, experiments, it has been observed that the vessels of the muscles do not respond passively to falls of pressure so readily as do the skin vessels. This can well be seen if the peripheral end of the vagus is stimulated simply to lower the arterial pressure (Fig. 14). A similar passive response of the skin vessels also shows itself in relation to asphyxia when an increase in total limb volume may accompany the rise of pressure caused by asphyxia, although the vessels of the muscles constrict (Fig. 7). This was somewhat unexpected and the experiment has been repeated many times.

DISCUSSION

The investigation indicates that the control of the vessels of the muscles is very similar to that of blood vessels in the rest of the body, that is, at rest they are under the tonic control of the vasoconstrictor centre, the action of which may be increased by haemorrhage, carotid occlusion, lactic acid injection and asphyxia and reduced by stimulation of the depressor fibres of the vagus. Several differences, however, were observed.

It has been shown that large falls of arterial pressure can be produced by depressor stimulation which apparently do not involve the vessels of the muscles. Similarly, large rises of blood pressure can be produced by clipping off the carotid sinus without any apparent changes in these vessels. How far this is due to a balanced effect between the vaso dilatation and the passive effects of a fall of blood pressure in the case of depressor stimulation and between vaso constriction and the rise of blood pressure in the case of carotid occlusion, it is difficult to assess, but the fact that more severe stimulation of the centre by asphyxia or cerebral anaemia produces contraction of the muscle vessels in spite of the rises of pressure, appears to indicate that the muscle vessels do not partake in the depressor reflexes as much as other vessels. It is of interest to remark that Ludwig & Cyon (1866), announcing their discovery of the depressor nerve, state that its action is practically confined to the region supplied by the splanchnic nerves, and according to Bayliss (1893) this was confirmed by Stelling (1867). Bayliss, however, found that the limb vessels

played a part in the response. It appears then that whether or not an effect on the limbs is seen depends on the exact conditions of the experiment.

The results generally suggest that *the different vessels respond in sequence* according to the degree of stimulation of the centre. In the present experiments it is evident that degrees of stimulation which affect the skin vessels may not affect those of the muscle, although the latter will respond if the stimulation is more severe. Thus the muscle vessels may not respond to the relatively mild release of the vasoconstrictor centre but do so if the release is more complete as occurs after the vagi are cut or if the centre is stimulated by asphyxia. Indeed it would seem reasonable if, as has been suggested, a function of the depressor reflexes is to maintain the capacity of the circulation and thereby a reserve of blood for use by the muscles in exercise. The suggestion is based on the finding that aortic depressor or carotid sinus stimulation lowers the venous pressure as well as the arterial (McDowall, 1935*a*, 1938), while in regard to sinus stimulation it has also been shown that a reduction of the depressor impulses from the sinus has the opposite effect, in the cat at least, and produces a rise of venous pressure and of cardiac output (Gollwitzer-Meier & Schulte, 1931; McDowall, 1934, 1935*a*, *b*).

Another interesting finding is the constrictor and dilatation response of denervated vessels to amounts of alkali and acid which do not affect the blood pressure. Incidentally, the observation emphasizes that the blood pressure may be a poor indicator of the changes occurring in the circulation. The constrictor effect of alkali on perfused vessels is well known. It was shown by Hemingway & McDowall (1926) that it was possible by means of alkali to produce in perfused vessels a tone dilatable by histamine while, on the other hand, Dale & Evans (1922) found that raising the pH of the blood to 8·5 by bicarbonate did not materially affect the blood pressure of the intact animal. We must, therefore, conclude either that the denervated vessels become abnormally sensitive to acid-base changes or that in the normal innervated vessels the peripheral constrictor effect of alkali is balanced by a depressant effect on the centre. In view of the finding by Dale & Richards (1918) that denervated vessels are hypersensitive to adrenaline and histamine once they have recovered their tone, the former explanation appears most likely, and it may now be suggested that this hypersensitivity to alkali may indeed be *the factor responsible for the normal recovery of tone* after denervation as in the perfusion experiments of Hemingway & McDowall (1926) and of McDowall (1928*a*). This suggestion is of considerable practical importance as it is found that after sympathectomy in man, the increased flow produced decreases after a few days (Learmonth, 1949). There is no doubt that, after denervation, the adrenaline dilator response which usually returns after a few hours may be made to do so almost immediately if alkali is given to increase peripheral

vascular tone. Too much alkali, however, abolishes even the normal dilator response produced by adrenaline on the muscle vessels.

The occurrence of a reduced blood flow from the limb veins when depressor stimulation from the carotid sinus is reduced without a corresponding change in volume suggests that the blood may even be trapped temporarily in muscles by the constriction of the venules, the function of which is not understood. The advantage of having such a mechanism at the very beginning of exercise when reduced activity of the depressor reflexes may occur to permit a rise of arterial pressure, is obvious. Its association with a general rise of venous pressure due to diminished capacity of the circulation suggests that the venule constriction may be peculiar to muscles. We can well believe that the venules do not open up until metabolites accumulate, but further evidence on this point will be sought. Another explanation might be suggested—namely that the lack of change in limb volume was the result of a combination of an active constriction of the arterioles, as shown by the reduction of venous flow, and passive dilatation of the venules as a result of the rise of venous pressure. This explanation, however, necessitates the possibility that the venules differ from the veins in not responding to increased activity of the vasoconstrictor centre. Donegan (1921) has shown that the veins normally respond to such increased activity, indeed the increased venous pressure is in part due to this.

The response, presumably passive, of the skin vessels to changes in blood pressure and in the presence, sometimes, as in asphyxia, of an opposite response in the muscle vessels is of some interest. It has long been suspected that the skin vessels were more sensitive to falls of internal pressure than those of the muscles for, in an ordinary faint, the skin vessels constrict and cause skin pallor at a time when generalized loss of vascular tone elsewhere leads to a fall of blood pressure and unconsciousness. On the other hand, the dilatation of the skin vessels in anger or asphyxia when the blood pressure is raised in man is well recognized. The asphyxial response may represent a vestigial reaction of the vessels of lower animal forms in which the blood is aerated through the skin as in frogs.

It was also demonstrated most clearly that both adrenaline and sympathetic section cause a dilatation of muscle vessels, a fact which recalls the old controversy as to whether, therefore, the substance liberated by the sympathetic can be adrenaline. If this were so, it might be expected that a dose of adrenaline could be found which would cause a return of the tone of the vessel which would nevertheless be dilated by small doses, as in perfusion experiments. Noradrenaline, kindly supplied to me by Prof. H. Barcroft, who witnessed some experiments, also produces a dilatation of the vessels of the muscles, so the whole problem still remains open.

SUMMARY

1. Plethysmographic studies of skinned hind-limbs in cats indicate that the vessels of the muscles, although under similar control to that of other vessels, are not so sensitive to depressor reflexes.

2. The vessels are tonically constricted by fibres of the lumbar sympathetic cord, in spite of the fact that they may be caused to dilate by injected adrenaline or noradrenaline.

3. They are also less sensitive to changes of internal pressure than are those of the skin and may react in quite an opposite direction.

4. When denervated, they respond to changes in the pH of the blood which may not affect the general blood pressure.

5. It is suggested that the venules may dam up blood in muscles.

6. Saline solution injected intravenously is very rapidly taken up by the muscles.

I have much pleasure in thanking Messrs Parke Davies for the supply of adrenaline used in some of these experiments.

REFERENCES

Barcroft, H. & Edholm, O. (1945). *J. Physiol.* **104**, 161.
Bayliss, W. M. (1893). *J. Physiol.* **14**, 303.
Dale, H. H. & Evans, C. L. (1922). *J. Physiol.* **56**, 125.
Dale, H. H. & Richards, A. N. (1918). *J. Physiol.* **52**, 110.
Donegan, J. F. (1921). *J. Physiol.* **55**, 226.
Gollwitzer-Meier, Kl. & Schulte, H. (1931). *Pflügers Archiv.* **229**, 264.
Hartman, F. A. & Fraser, L. M. (1917). *Amer. J. Physiol.* **44**, 353.
Hemingway, A. & McDowall, R. J. S. (1926). *J. Physiol.* **62**, 166.
Hill, L. (1896). *The Cerebral Circulation.* London: Churchill.
Hoskins, R. G., Gunning, R. E. L. & Berry, E. L. (1916). *Amer. J. Physiol.* **41**, 513.
Jarisch, A. (1925). *J. Physiol.* **40**, 419.
Learmonth, J. R. (1949). (Private communication.)
Ludwig, C. & Cyon, E. von (1866). *Ber. Verh. Sächs Ges. Leip.* **18**, 307.
McDowall, R. J. S. (1924). *J. Physiol.* **59**, 41.
McDowall, R. J. S. (1928*a*). *J. Physiol.* **65**, 25.
McDowall, R. J. S. (1928*b*). *J. Physiol.* **66**, 17*P*.
McDowall, R. J. S. (1930). *J. Physiol.* **69**, 11*P*.
McDowall, R. J. S. (1932). *J. Physiol.* **77**, 21*P*.
McDowall, R. J. S. (1934). *J. Physiol.* **82**, 4*P*.
McDowall, R. J. S. (1935*a*). *J. Physiol.* **84**, 24*P*.
McDowall, R. J. S. (1935*b*). *J. Physiol.* **86**, 7*P*.
McDowall, R. J. S. (1938). *The Control of the Circulation of the Blood.* London: Longmans Green.
McDowall, R. J. S. (1942). *J. Physiol.* **100**, 17*P*.
Stelling, C. (1867). *Diss Dorpat.* (Quoted by Bayliss, 1893.)

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THE RESPONSES OF THE HEART RATE AND THE SYSTOLIC BLOOD PRESSURE TO A SERIES OF SYMPATHOMIMETIC AMINES IN THE UN-ANAESTHETIZED, ATROPINIZED BITCH

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The purpose of the present series of experiments was to discover whether common types of receptors for sympathomimetic amines were universally distributed throughout the cardiovascular system. The responses of the heart rate and the systolic blood pressure were selected for initial investigation. These two responses to each of a series of sympathomimetic amines have been measured in unanaesthetized, atropinized bitches before and after both sympathectomy and adrenalectomy.

The effect of cardiac denervation on the responses of the heart rate to adrenaline was studied by Burrett (1940) in anaesthetized cats. Observations were made, under pentobarbitone anaesthesia, during the first 29 post-operative days; an increased sensitivity to adrenaline was recorded. The work of Bülbring & Burn (1938) and of many others, reviewed by Burn (1938), has shown that anaesthesia may reverse the effect of sympathetic stimulation. In the work here described, anaesthesia has been avoided. The alterations produced by pentobarbitone anaesthesia in the responses of the heart rate and systolic blood pressure to sympathomimetic amines in normal, sympathectomized, and adrenalectomized dogs are to be reported elsewhere (Lockett, 1950*a*).

Selye (1936) reported that adrenalectomy lowered the tolerance of rats to large and repeated subcutaneous doses of adrenaline, but it was of interest to determine the effect of adrenalectomy on the responses of the heart rate and the systolic blood pressure to a group of sympathomimetic amines injected intravenously in doses producing submaximal effect.

METHODS

Mongrel bitches, those later sympathectomized and adrenalectomized resembling terriers, were equipped with carotid loops (van Leersum, 1911), and the corresponding carotid sinus was denervated (Verney & Vogt, 1938). Sympathectomy was performed as previously described (Lockett, 1949*a*). Adrenalectomy was carried out in two stages, 1 week apart, by the lumbar

route. Adrenalectomized dogs received normal diet; the replacement therapy given during the experiments recorded in this paper is listed in Table 5. The hormone requirement for maintenance of adrenalectomized-sympathectomized dogs was found to be approximately one-half that required by adrenalectomized animals (Lockett, 1949*b*).

Training. The bitches were first thoroughly accustomed to the blood-pressure cuff, to intravenous injections, and to the taking of electrocardiographic records. Subsequently, intravenous injections of two fixed weights of adrenaline, each in 0·4 ml. 0·9% NaCl, were given in random order,

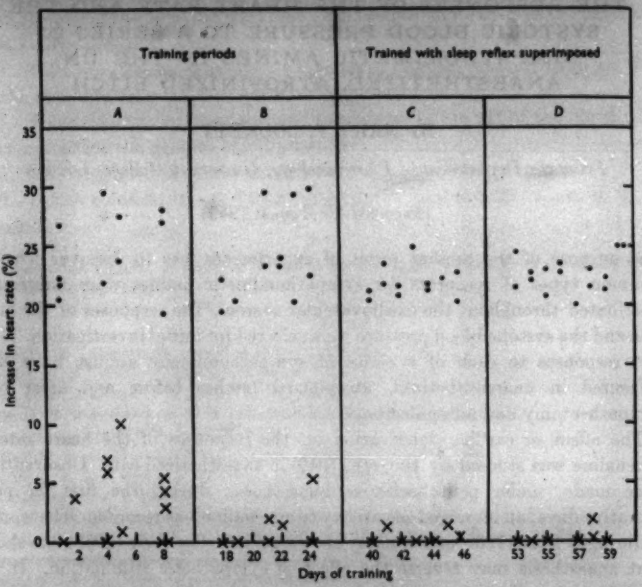

Fig. 1. Decreasing scatter of the day-to-day responses of the heart rate to the intravenous injection of 14 μg. L-adrenaline (dots), and 0·9% NaCl (crosses), for four periods, *A*, *B*, *C*, and *D* each of 1 week, during the training of bitch 6. Subcutaneous atropine sulphate, 6 mg., 45 min. before each experiment.

interspersed with saline injections, 0·4 ml., until the day-to-day scatter of observations on the magnitude of the heart-rate responses approached a constant minimum. The injections occupied, as nearly as possible, 2 sec. Fig. 1 shows the distribution of the heart-rate responses to the intravenous injection of 15 μg. adrenaline chloride and 0·9% NaCl, for 4 separate weeks during the training of a single bitch.

It was found that even after several months of practice, steady basal values of heart rate and systolic blood pressure were rarely maintained for more than 45 min. When conditioned sleep reflexes had been superimposed upon the previous training, however, several hours became available for the repeated comparison of the responses to the several amines on a single day. In Fig. 2 the systolic blood pressure and heart rate of an individual and typical dog during atropinized sleep are contrasted with those of the same animal during unatropinized conditioned sleep. Whereas, unatropinized, there was a steep fall in systolic blood pressure and heart rate during the first hour,

or more, of sleep, this was absent during full atropinization. In the fully atropinized sleeping dog, basal heart rates and systolic blood pressures were very steady for 2–3 hr., and showed only a very gradual decline.

Such dogs were not awakened by injections, by determinations of blood pressure, or by the making of electrocardiographic records from which the heart rates have been determined.

Atropinization. Atropine sulphate, 1 mg./kg. subcutaneously, was badly tolerated by conscious dogs. Symptoms encountered in ten dogs after this dose have been: non-co-operation (10), vomiting (8), tremors (8), hysterical yapping or crying (4), extreme nervousness (4), restlessness (7), which amounted to a desire to run blindly in 2. Drowsiness was usually intermingled with the other symptoms, but formed the main feature in one animal.

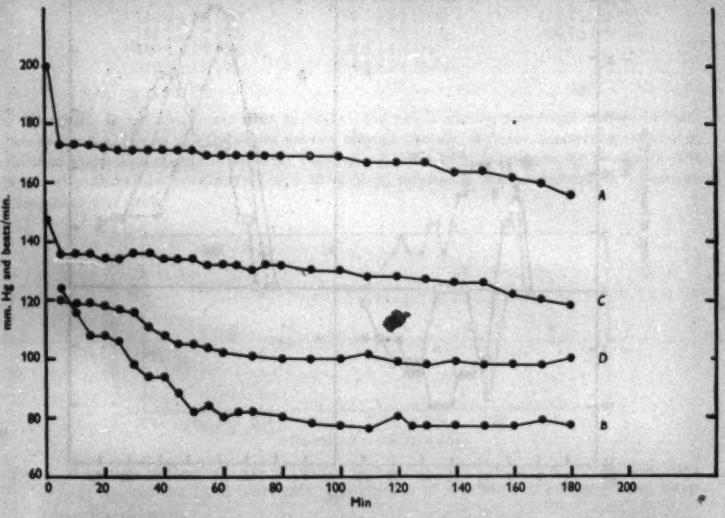

Fig. 2. Systolic blood pressure and heart rate of bitch 6, during the first 3 hr. of conditioned sleep, atropinized and unatropinized. *A*, heart rate, after atropine; *B*, heart rate without atropine; *C*, blood pressure after atropine; *D*, blood pressure without atropine.

Eight of the ten dogs tolerated 0·8 mg./kg. atropine sulphate, subcutaneously, sufficiently well to permit the onset of fairly strong conditioned sleep reflexes. The other two dogs tolerated 0·6 mg./kg. atropine sulphate similarly administered. These weights of atropine, given 45 min. before experiments, were found sufficient to produce maximum increases in heart rate in the response to the injection of fixed weights of the several sympathomimetic amines studied. In long experiments, a further 2 mg. atropine sulphate were injected subcutaneously each hour.

The dose of atropine sulphate required to produce maximum responses of the heart rate after the intravenous injection of fixed weights of these sympathomimetic amines were not altered by sympathectomy, in the four dogs so examined. When the three sympathectomized and one normal dog had been adrenalectomized, an increased dose of 0·2 mg./kg. atropine sulphate, and an increase of 15 min. in the time allowed for absorption, was required in each animal to produce full atropinization.

Larger amounts of atropine were required to convert the bradycardia produced by the intravenous injection of noradrenaline and β-phenylethylamine into tachycardia, than were needed to produce a similar effect on the responses to adrenaline and tyramine. Fig. 3 illustrates this point; curves relating increase in heart rate to time after the intravenous injection of fixed weights of three amines, are shown, for two doses of atropine, 2 and 4 mg., in one dog. Each set of curves was drawn through the means of observations made on 3 separate days.

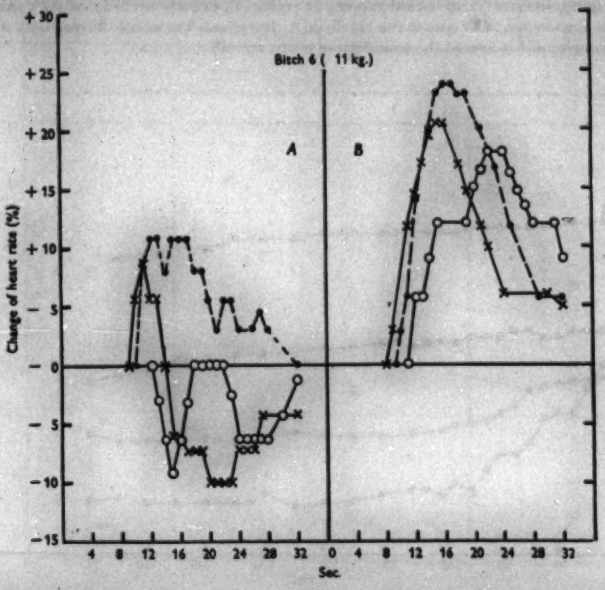

Fig. 3. Mean responses of bitch 6 to fixed weights of three amines, equally active on the heart rate at full atropinization. *A*, after 2 mg. atropine sulphate subcutaneously; *B*, after 4 mg. atropine sulphate; see text. ×—×, 10 μg. DL-noradrenaline; ●---●, 10 μg. L-adrenaline; ○—○, 1·2 mg. β-phenylethylamine.

The range of basal heart rates and systolic blood pressures encountered in individual dogs, sleeping fully atropinized, from day to day, was considerable, as is shown in Table 1. However, no correction for basal heart rate or systolic blood pressure, as possibly affecting the size of response, was applied. Fig. 4 demonstrates that even in a dog which showed an unusually wide range of basal heart rate from day to day, no correlation could be found between the size of the response and the basal heart rate. Any correction which might have been applied was evidently insignificant when compared to the variation in the responses of the dog from day to day.

Injections were made aseptically. Atropine sulphate was given subcutaneously, 4 mg./ml. 0·9 % NaCl. Amine salts in 0·4 ml. 0·9 % NaCl were injected into malleolar veins, the injections occupying as nearly as possible 2 sec.

Measurements of systolic blood pressure were made from carotid loops.

TABLE 1. Basal heart rates and systolic blood pressures of fully atropinized sleeping bitches, normal, and after sympathectomy and adrenalectomy

Dog	Normal	Sympathectomized	Sympathectomized and adrenalectomized
		Basal heart rates (beats/min.)	
2	174·3±20·6 (25)	164·4±17·9 (107)	168·3±18·5 (102)
3	177·0±24·0 (22)	162·3±14·9 (101)	157·2±17·4 (78)
1	176·3±18·4 (22)	158·3±16·6 (62)	—
5	172·6±15·1 (99)	—	—
4	187·4±14·8 (32)	166·7±13·9 (31) (adrenalectomized)	163·5±18·6 (39)
		Basal systolic blood pressure (mm. Hg)	
2	149·4±2·0 (86)	120·1±3·2 (125)	114·4±4·6 (52)
3	139·2±1·3 (80)	118·2±3·1 (108)	106·7±5·1 (48)
1	160·1±1·7 (63)	135·8±1·8 (46)	—
5	152·0±1·0 (59)	134·6±2·4 (120)	—
4	—	—	—

Heart rate. In ten atropinized sleeping bitches, the curves relating percentage increase in heart rate to the log dose of the respective amines, showed two almost linear intersecting sections of different slope. This change in slope lay between a 7 and a 14 % increase in the heart rate. The upper slope remained constant up to a 33 or 35 % increase in heart rate, and was employed throughout this work.

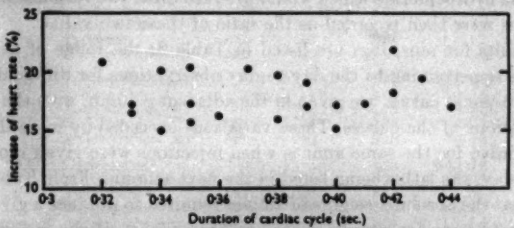

Fig. 4. Responses of the heart rate of bitch 6, fully atropinized, obtained on 22 successive days, plotted against the basal heart rate. The absence of correlation is evident. Dose, 10 μg. adrenaline chloride.

Two or three weights of each amine, which produced well-spaced submaximal responses greater than a 15 % increase in heart rate, were selected for each animal. The chosen doses of DL-noradrenaline, L-adrenaline, tyramine and β-phenylethylamine were given intravenously in random order, interspersed with saline injections, on 4–6 days of each week, for a number of weeks. The time interval between injections was 7 min. each day. Because amphetamine tended to produce potentiation of the responses to other sympathomimetic amines, and in four of eight dogs showed tachyphylaxis when given repeatedly at 7 min. intervals, injections of this amine were given after the other amines had been tested, and the time interval was lengthened to 12 min. Amphetamine was not used on days when a single dose of ephedrine was given, at the end of the experiment. Repeated similar doses of β-phenylethylamine at 7 min. intervals have frequently produced decreasing responses of the heart rate. It was decided not to exclude β-phenylethylamine from the series of amines given in random order on this account; neither was it considered advisable to alter the time interval between injections of a random series.

Systolic blood pressures. Curves relating the rises of systolic blood pressure in mm. Hg. to the log doses of the respective amines were not markedly sigmoid in fully atropinized sleeping bitches.

Linearity of these log dose-response curves extended upward over a range of more than 100 mm. Hg, from responses of the order of 15–20 mm. Hg. Amines were tested for their effect on systolic blood pressure much as is described above for the measurement of the changes they produced in the heart rate. Duplicate measurements were made each day.

The mean dose-response curves, and the variations in the responses to fixed weights of each amine, both from day to day, and on individual days, were computed from the collected measurements.

RESULTS

The responses of the heart rate

The mean curves relating percentage increase in heart rate to log dose, expressed as amine hydrochloride, were determined for a series of sympathomimetic amines in eight fully atropinized sleeping bitches, as described above. The slopes of the dose-response curves for the different amines in the individual dogs were not parallel. In making comparisons between the activity of these amines it was therefore necessary to state both the positions and the slopes of the linear curves. This was most readily effected by the arbitrary selection of two response levels, a 10 and a 25% increase in heart rate, and then by determining from the mean curves obtained for each amine in each dog the two weights of each amine hydrochloride which would produce these two responses. The slopes of the lines were then recorded as the ratio of these two values.

The results for nine dogs are listed in Table 2; the range of the standard deviations appertaining to the day-to-day observations for different points on the dose-response curves are given in the adjacent column, with the number of determinations of the curves. These variations exceeded by more than tenfold those recorded for the same amines when injections were given repeatedly on the same day, the latter being listed in the next column. From Table 2 it may be seen that the absolute weights of amines required to produce a given increase in heart rate were not simply related to the weights of the individual dogs. The hydrochlorides of L-adrenaline and DL-noradrenaline had by weight approximately equal activity on the heart rate in fully atropinized sleeping bitches, and the slopes of the dose-response curves were very similar. The order of the other amines, arranged in decreasing strength of action on the heart rate, differed from dog to dog only because of the great variation encountered between the sensitivity of the individual animals to amphetamine.

Part I

The position and slope of the mean dose-effect curves for the different sympathomimetic amines are characteristics of the individual atropinized animal

Comparison of the mean dose-response curves obtained for the individual animals (Figs. 5, 8 and 9) showed that, as far as the heart-rate responses to sympathomimetic amines were concerned, the differences between individual bitches were so great as to preclude the use of a small group of control animals for comparison with those which had undergone operative procedures. It was

TABLE 2. The responses of the heart rate to sympathomimetic amines in sleeping atropinized bitches

Dog	Weight (kg.)	Amine hydrochloride in order of potency	μg. amine hydrochloride producing % increase heart rate		Slope of curve ratio 25 ÷ 10 % response	Standard deviation of % response			
						Separate days		Single days	
			25 %	10 %		6	N	6	N
6 (ix. 48)	11·1	L-Adrenaline	15·8	6·3	2·51	1·3–2·7	28	0·11	10
		DL-Noradrenaline	16·8	5·7	2·94	1·2–0·9	12	0·04	12
		Tyramine	960	670	1·43	2·7–3·1	9	0·08	8
		Amphetamine	1010	710	1·42	0·7–1·0	12		
		β-Phenylethylamine	1660	960	1·73	2·5–3·0	21	0·10	10
6 (iii. 49)	11·2	L-Adrenaline	14·2	6·2	2·29	2·0–4·1	33		
		DL-Noradrenaline	16·4	6·1	2·69	1·0–2·0	24		
		Tyramine	968	662	1·46	0·8–3·0	9		
		Amphetamine	1060	680	1·56	0·8–1·0	12		
		β-Phenylethylamine	1540	950	1·62	2·8–3·5	21		
7 (viii. 48)	11·4	L-Adrenaline	12·8	7·7	1·66	1·7–1·9	18		
		Amphetamine	905	690	1·31	2·1–2·5	12		
		Tyramine	1450	840	1·73	1·5–3·5	18		
		β-Phenylethylamine	1560	880	1·77	1·8–2·1	18		
7 i. 49)	11·2	L-Adrenaline	12·8	7·9	1·62	1·3–1·6	18	0·10	12
		Amphetamine	900	680	1·32	2·0–2·9	12	0·13	12
		Tyramine	1380	845	1·63	1·1–3·8	12	0·11	12
		β-Phenylethylamine	1550	860	1·80	1·7–2·4	12	0·14	12
1	9·0	DL-Noradrenaline	18·2	7·6	2·39	0·7–0·7	9		
		L-Adrenaline	21·3	7·75	2·75	0·9–1·1	12		
		Amphetamine	965	570	1·69	1·2–1·6	12		
		Tyramine	1800	640	2·81	1·5–1·3	12		
		β-Phenylethylamine	2460	800	3·08	1·4–1·8	12		
1	9·1	DL-Noradrenaline	19·6	7·4	2·65	0·5–0·75	12	0·05	7
		L-Adrenaline	23·2	7·6	3·05	0·6–1·0	15	0·04	7
		Amphetamine	970	560	1·73	1·3–1·6	8		
		Tyramine	1800	640	2·80	1·0–1·1	8	0·08	7
		β-Phenylethylamine	2480	780	3·18	0·6–0·9	8	0·11	7
4	9·1	L-Adrenaline	23·0	5·4	4·26	0·15–0·8	18	0·11	6
		DL-Noradrenaline	26·0	5·0	5·20	0·3–0·2	25	0·10	6
		Tyramine	1800	450	4·00	1·5–0·9	15	0·14	6
		Amphetamine	1380	610	2·26	1·5–1·8	12		
		β-Phenylethylamine	2530	760	3·33	1·2–1·5	15	0·21	6
5	10·7	L-Adrenaline	10·8	5·2	2·08	1·9–2·1	40	0·15	6
		DL-Noradrenaline	11·0	5·4	2·04	0·1–0·4	30	0·10	6
		Tyramine	850	315	2·70	1·8–2·4	18	0·17	6
		Amphetamine	960	480	2·00	0·5–1·5	18		
		β-Phenylethylamine	1160	455	2·55	3·3–3·7	11	0·34	6
3	10·1	DL-Noradrenaline	16·25	9·7	1·68	0·9–1·1	9	0·01	18
		L-Adrenaline	18·3	10·4	1·76	0·8–0·9	9	0·02	14
		Amphetamine	13·0	800	1·63	0·8–1·1	3		
		Tyramine	1820	1010	1·80	2·5–2·1	6	0·12	7
		β-Phenylethylamine	2300	1150	2·19	2·5–3·2	6	0·14	7
2	11·7	DL-Noradrenaline	16·4	8·4	1·95	1·9–2·0	12	0·09	12
		L-Adrenaline	19·4	9·6	2·02	2·3–2·5	24	0·11	16
		Amphetamine	1180	760	1·55	2·0–2·5	6		
		Tyramine	1500	670	2·24	2·0–2·3	9	0·16	9
		β-Phenylethylamine	1625	900	1·81	1·3–1·5	12	0·18	9
8	14·9	L-Adrenaline	14·5	6·3	2·30	1·3–1·6	22	0·08	12
		DL-Noradrenaline	15·0	6·0	2·50	1·9–2·0	18	0·05	12
		Tyramine	1440	500	2·88	1·3–0·8	15	0·07	7
		Amphetamine	1540	945	1·63	1·9–2·1	15		
		β-Phenylethylamine	2370	1150	2·06	1·1–2·9	15	0·17	7
9	16·4	L-Adrenaline	14·0	8·1	1·73	1·0–2·1	18	0·12	7
		DL-Noradrenaline	14·0	8·1	1·73	0·7–1·0	18	0·04	7
		Amphetamine	1740	585	2·97	1·5–2·0	10		
		Tyramine	2070	680	3·04	1·5–2·0	14	0·15	7
		β-Phenylethylamine	2300	1160	1·98	0·9–1·3	16	0·21	7

therefore imperative to determine whether the responses of any one bitch to a series of sympathomimetic amines might be expected to remain reasonably constant for a period of months.

Two sets of values for the mean dose-response curves are given for each of the first three bitches listed in Table 2. These two determinations were separated by intervals of 6, 4 and 2 months respectively, and are illustrated in Fig. 5.

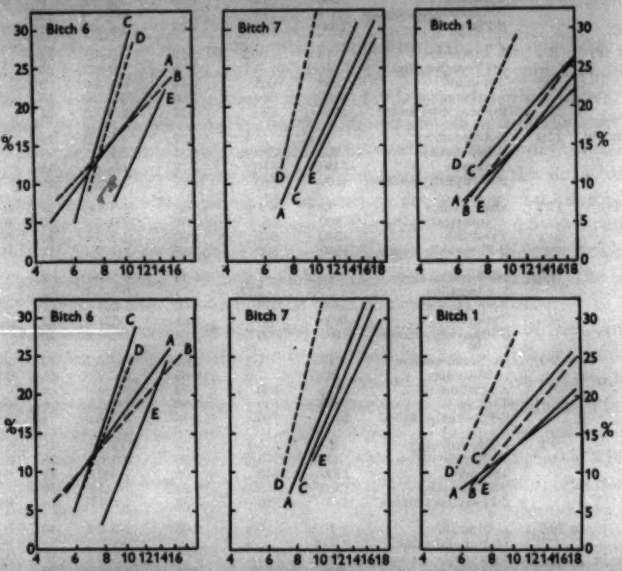

Fig. 5. Remarkable similarity exists between the two sets of curves relating percentage increase in heart rate (ordinate) to log dose of amine hydrochloride, for bitches 6, 7 and 1 (Table 2). Time interval between the two sets of observations 6, 4, and 2 months respectively. Abscissa: weight amine hydrochloride, adrenaline (*A*) and noradrenaline (*B*) in μg., tyramine (*C*), amphetamine (*D*), β-phenylethylamine (*E*) mg. ÷10.

Between these two sets of estimations the first-conditioned sleep reflex in each animal was destroyed, and a second sleep reflex dependent on different stimuli, was induced. The first dog listed in Table 2 and Fig. 5, had, in the interval, undergone three major operations, for double renal explantation (Lockett, 1949*c*). The remarkable similarity of the two sets of curves obtained for the individual animals, and the striking differences between the curves relating to the separate bitches, at once suggested that the mean dose-response curves obtained were in fact normal characteristics for the individuals.

It was concluded from the foregoing observations, that, provided strict adherence was maintained to the experimental methods previously described, comparison might justly be made between the mean dose-response curves to these sympathomimetic amines, in any one animal before and after operation, e.g. sympathectomy.

Part II

The effects of sympathectomy

After the mean curves, relating percentage increase in heart rate to log dose of amine hydrochloride, had been determined for five amines in dogs 1–3 (Table 2), the sympathetic chains were removed from just above the stellate ganglia, downward into the pelvis. The operation was performed in three stages, occupying 4–6 weeks: recovery in each case was uninterrupted. Four to five weeks from the time of the mid-stage operation the systolic blood pressures of these dogs had returned to within a few mm. Hg of the pre-operative values, when sleeping unatropinized (Lockett, 1949*a*), but remained for 20 weeks of observation lower than the corresponding control values when sleeping fully atropinized (see Table 1).

TABLE 3. The responses of the heart rate to L-adrenaline and DL-noradrenaline, in fully atropinized bitches after sympathectomy

	L-Adrenaline hydrochloride					DL-Noradrenaline hydrochloride					
	μg. producing rise in heart rate		Slope of log dose-response curve (ratio 25÷10%)	Standard deviation of response (%)	N	μg. producing rise in heart rate		Slope of log dose-response curve (ratio 25÷10%)	Standard deviation of response (%)	N	Weeks from mid-operation
Dog	25%	10%				25%	10%				
3	5·3	1·9	2·79	3·6–2·0	11	15·0	4·0	3·75	2·6–1·4	11	5–7
	5·5	2·05	2·68	2·6–2·1	11	10·5	3·9	2·69	2·4–2·1	11	10–12
	6·4	2·05	3·12	2·1–1·1	10,	9·3	4·3	2·16	1·9–1·0	10	13–17
2	7·1	2·0	3·55	3·5–2·5	17	8·1	2·4	3·38	3·0–2·5	17	3–6
	4·6	2·65	1·73	3·4–3·2	20	7·7	2·4	3·21	2·1–1·9	20	8–10
	5·0	2·6	1·92	2·4–1·2	20	5·8	2·5	2·32	2·4–2·0	20	16–20
	5·5	2·6	2·2	2·5–1·7	22	6·4	2·5	2·56	1·3–1·1	22	20–24
1	7·0	2·05	3·41	2·0–1·6	6	11·5	2·65	4·34	1·8–1·2	6	3
	4·6	2·2	2·09	2·0–1·7	6	10·7	3·25	3·29	1·9–1·2	6	6
	4·8	2·3	2·08	1·4–1·0	6	8·4	3·6	2·33	1·8–1·4	6	8

Adrenaline and noradrenaline. The changes in the responses of the heart rate to L-adrenaline and to DL-noradrenaline were followed more closely than were the responses to the other sympathomimetic amines studied. In Fig. 6 are shown the positions and slopes of the mean curves relating log dose and percentage response for these two amine hydrochlorides, in three dogs, before operation, and for successive intervals of several weeks, timed from the mid-operative stage of sympathectomy. In each dog, sensitivity to L-adrenaline was increased more than to DL-noradrenaline: whereas the slopes of the dose-response curves for noradrenaline, and sensitivity to this amine increased progressively in the first 4 months after sympathectomy, a maximum sensitivity and curve slope for the action of adrenaline on the rate of the atropinized heart was encountered in all three dogs at about the 50th day from the mid-operative

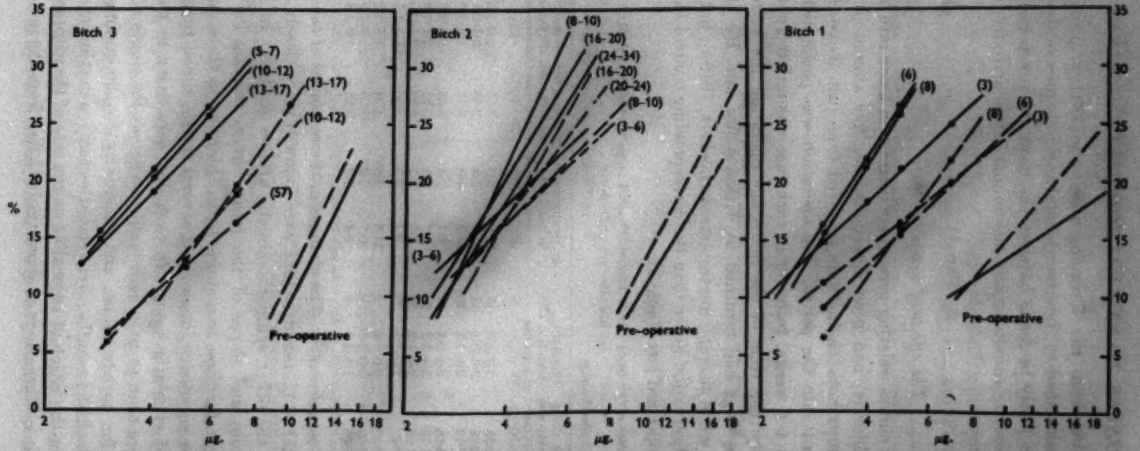

Fig. 6. Mean dose-response curves for bitches 3, 2, and 1, relating percentage increase in heart rate (ordinate) to weight of amine hydrochloride (abscissa), for L-adrenaline (—●—) and DL-noradrenaline (— - ●- - —), before and after sympathectomy. The figures in brackets indicate the post-operative weeks during which the measurements were made.

stage of sympathectomy (Fig. 7). These results are summarized in Table 3. The weights of amine hydrochloride in micrograms which produced a mean increase in heart rate of 25 and 10% are listed. The slopes of the dose-response curves are expressed as a ratio of these two weights. The extreme values of the standard deviations of the responses to each of the multiple fixed weights of amine hydrochloride, used in the determination of these mean curves, are then given, followed by the number of separate curve determinations.

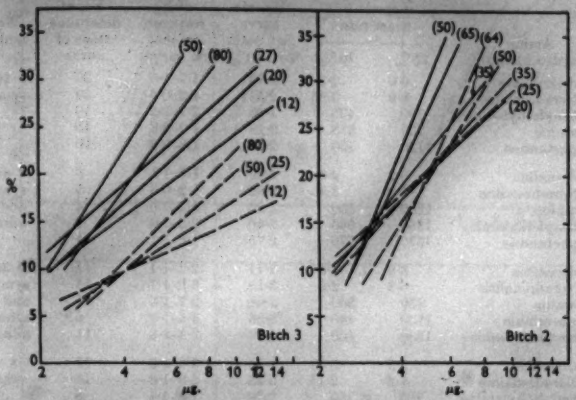

Fig. 7. Changes in the slopes of the dose-response curves of L-adrenaline (——) and DL-noradrenaline (---) in their effect on the heart rate of two atropinized bitches, after sympathectomy. The figures in brackets indicate the post-operative day on which each curve was determined. Abscissa and ordinate as in Fig. 6. The slope of the noradrenaline curves increased gradually to the 80th and 65th days; the adrenaline curves showed a maximum slope at about the 50th post-operative day.

Tyramine, β-phenylethylamine, and amphetamine. The changes which followed sympathectomy, in the responses of the heart rate to β-phenylethylamine, tyramine, and amphetamine, in atropinized sleeping bitches are illustrated in Fig. 8, and are summarized in Table 4. Removal of the sympathetic chains, including the stellate ganglia downward into the pelvis, increased the responses of the heart rate to both β-phenylethylamine and tyramine, and reduced the responses to amphetamine. Two bitches, nos. 1 and 2, in Table 4, were repeatedly examined in the 6th–8th weeks after sympathectomy. The pre-operative dose-response curves for these animals are given in Table 2 and Fig. 8. In Fig. 8 it is shown that whereas sympathectomy decreased the sensitivity of the heart-rate responses to amphetamine, there was an increase in sensitivity to β-phenylethylamine which exceeded the increase in sensitivity toward tyramine. Bitch 3 was twice examined during this post-operative period; the observations made confirmed those recorded for bitches 1 and 2.

TABLE 4. The responses of the heart rate to sympathomimetic amines in sleeping atropinized dogs after sympathectomy and adrenalectomy

Dog	Amine hydrochloride	Mean dose-response curve: μg. amine HCl injected intravenously producing increase in heart rate 25%	10%	Slope of log dose-response curve (wt. ratio 25÷10%)	Standard deviation of % response (points on curve)	No. of determinations of curve	Weeks from mid-operative stages
3	L-Adrenaline	6·0	2·2	2·73	1·7–1·0	20	10–16 after sympathectomy
	DL-Noradrenaline	9·9	3·95	2·51	1·2–0·6	20	
	β-Phenylethylamine	700	430	1·63	2·7–2·2	10	
	Tyramine	730	335	2·18	1·0–0·8	10	
	Amphetamine	1120	430	2·60	1·8–0·9	10	
	L-Adrenaline	6·2	2·6	2·39	1·5–1·1	5	21 weeks after sympathectomy and 2 weeks after adrenalectomy
	DL-Noradrenaline	8·9	3·4	2·62	1·2–0·8	5	
	Tyramine	1200	530	2·26	1·2–1·0	4	
	β-Phenylethylamine	1380	560	2·46	2·2–1·0	4	
	Amphetamine	1570	570	2·75	1·6–1·1	4	
	L-Adrenaline	3·9	1·8	2·17	2·2–1·1	11	29–34 weeks after sympathectomy and 10–15 weeks after adrenalectomy
	DL-Noradrenaline	6·3	2·0	3·15	3·0–1·7	11	
	Tyramine	750	360	2·08	2·7–1·5	11	
	Amphetamine	1700	440	3·86	3·6–1·9	6	
	β-Phenylethylamine	1880	460	4·08	3·3–2·8	11	
2	L-Adrenaline	4·3	2·33	1·85	3·7–2·3	17	6–8 weeks after sympathectomy
	DL-Noradrenaline	7·2	2·15	3·35	2·0–1·6	17	
	β-Phenylethylamine	725	260	2·79	1·8–1·4	8	
	Tyramine	575	285	2·02	2·0–1·7	8	
	Amphetamine	1270	350	3·63	2·4–1·6	8	
	L-Adrenaline	5·5	2·6	2·12	2·5–1·7	16	20–24 weeks after sympathectomy
	DL-Noradrenaline	6·4	2·5	2·56	1·0–0·8	16	
	Tyramine	760	340	2·24	1·9–1·0	16	
	Amphetamine	1400	310	4·52	0·8–0·6	12	
	β-Phenylethylamine	1800	390	4·62	2·9–2·7	16	
	L-Adrenaline	5·8	2·65	2·19	2·8–1·8	10	28–32 weeks after sympathectomy and 3–7 weeks after adrenalectomy
	DL-Noradrenaline	8·0	4·0	2·00	4·8–3·5	10	
	Tyramine	870	550	1·58	1·4–0·8	6	
	Amphetamine	1360	480	2·83	1·6–0·4	6	
	β-Phenylethylamine	2000	580	3·45	1·1–1·0	6	
1	L-Adrenaline	4·7	2·3	2·04	1·7–1·1	6	7–8 weeks after sympathectomy
	DL-Noradrenaline	9·0	3·4	2·65	1·0–0·8	6	
	β-Phenylethylamine	665	380	1·75	2·9–2·0	7	
	Tyramine	700	395	1·77	1·9–1·4	6	
	Amphetamine	1700	450	3·78	1·3–0·8	4	
4	DL-Noradrenaline	12·6	3·6	3·50	0·8–0·4	9	6–10 weeks after adrenalectomy
	L-Adrenaline	15·0	3·9	3·85	1·7–1·4	9	
	Tyramine	610	260	2·35	1·0–0·7	4	
	Amphetamine	820	390	2·10	1·6–1·0	4	
	β-Phenylethylamine	950	310	3·06	1·8–1·3	4	
	L-Adrenaline	6·4	1·9	3·37	1·7–1·5	5	5 weeks after sympathectomy and 17 weeks after adrenalectomy
	DL-Noradrenaline	6·8	2·0	3·40	1·6–1·3	5	
	Tyramine	910	330	2·76	1·2–1·1	5	
	β-Phenylethylamine	1900	480	3·96	2·1–1·4	5	
	Amphetamine	2300	520	4·42	1·5–1·4	5	

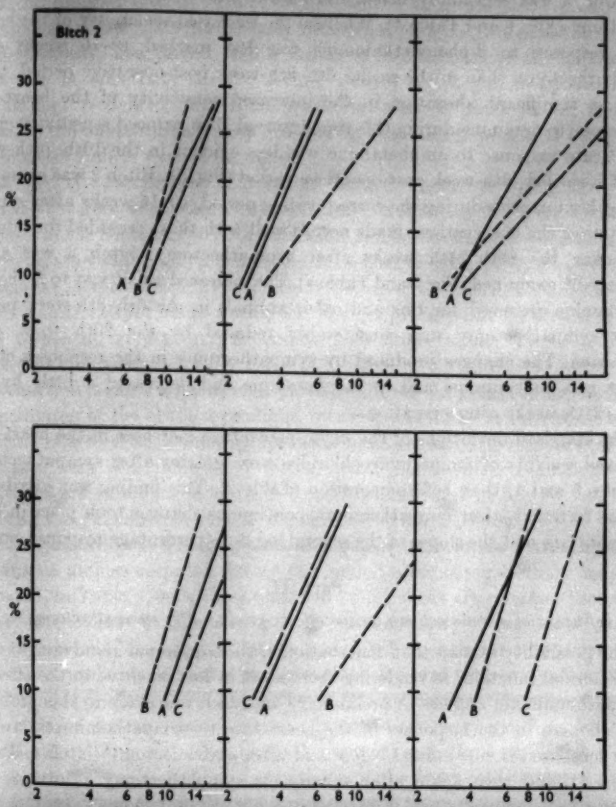

Fig. 8. Relation between percentage increase in heart rate and dose of amine hydrochloride, for tyramine (*A*), amphetamine (*B*), and β-phenylethylamine (*C*) in two atropinized bitches, before and after sympathectomy. Ordinates and abscissae, as in Fig. 5. The upper charts relate to bitch 2. Left, operative. Centre, 6–8 weeks after sympathectomy; the increased sensitivity of the responses of the heart rate to β-phenylethylamine exceeds that to tyramine; sensitivity to amphetamine is reduced. Right, 20–24 weeks after sympathectomy. Whereas the earlier changes in sensitivity to β-phenylethylamine and amphetamine were reduced, the increase in sensitivity toward tyramine was well maintained. The lower three charts relate to bitch 3 and, from left to right, show similar curves before sympathectomy, 6–8 weeks and 10–16 weeks after sympathectomy.

Bitch 3 was repeatedly tested during the 10th–16th weeks after sympathectomy (Fig. 8 and Table 4). Whereas the increased sensitivity of the heart-rate response to β-phenylethylamine was less marked 10–16 weeks after sympathectomy than in the earlier 6th–8th-week post-operative period, there was no significant alteration in the increased sensitivity of the heart-rate responses to tyramine during this time interval. The reduced sensitivity of the heart-rate response to amphetamine was less evident in the 10th–16th-week than in the 6th–8th-week post-operative period (Fig. 8). Bitch 2 was examined at weekly intervals during the corresponding period, 10–16 weeks after sympathectomy; the observations made accord well with those recorded for bitch 3.

During the 20th–24th weeks after sympathectomy, bitch 2 was again repeatedly examined (Fig. 8 and Table 4). The increased sensitivity to β-phenylethylamine observed for this and other animals in the 6th–8th-week period after sympathectomy was considerably reduced by the 20th week after operation. The changes produced by sympathectomy in the responses of the heart rate to tyramine and to amphetamine had decreased a little by the 20th–24th weeks after operation.

The standard deviations of the mean percentage increases in the heart rate to fixed weights of amine hydrochlorides were greater after sympathectomy (Tables 3 and 4) than before operation (Table 2). This finding was attributed to the fact that, after sympathectomy, continuous changes took place in both the positions and the slopes of the several log-dose percentage response curves.

Part III

The influence of adrenalectomy on the effects produced by sympathectomy

The possibility existed that denervation of the suprarenal glands might alter their normal function, so rendering them more or less sensitive to the effect of sympathomimetic amines. A preliminary approach was made to this problem. The changes in the responses of the heart rate to sympathomimetic amines were measured in one animal before and after adrenalectomy (bitch 4, Tables 2 and 4), and then again after subsequent sympathectomy. Further, two

Table 5. Treatment and analysis of blood and circulatory state of adrenalectomized dogs during experiments

Dog	Weight (kg.)	Suprarenal extract (ml./day)	Blood analysis			Circulation		Operated state (S = sympathectomized A = adrenalectomized)
			Plasma chloride (mg./100 ml.)	Non-protein N_2 (mg./100 ml.)	Haematocrit (%)	Time leg to leg (sec.)	Systolic blood pressure unatropinized (mm. Hg)	
3	10·0–10·2	0·5 ml. 'Mayo'	588·2 ± 2·9 (16)	41·1 ± 0·6 (12)	45·8 ± 0·4 (21)	45–50	91·3 ± 0·6 (14)	S + A
2	11·6–11·8	1·0 ml. Eucortone	597·4 ± 5·6 (14)	32·8 ± 0·9 (12)	46·2 ± 0·4 (12)	35–40	108·6 ± 1·2 (9)	S + A
4	9·1–9·2	2·0 ml. 'Mayo'	580·8 ± 1·3 (19)	31·1 ± 0·8 (18)	47·4 ± 0·8 (16)	40–45	114·4 ± 0·8 (11)	A
	9·0–9·4	0·6 ml. 'Mayo'	585·6 ± 3·1 (12)	30·7 ± 0·5 (12)	46·7 ± 1·2 (12)	40–45	112·6 ± 1·4 (6)	S + A

sympathectomized bitches were adrenalectomized when the rapid changes in the responses of the heart rate to sympathomimetic amines, which had followed sympathectomy, had passed, and a steady slow decline from the maximum alterations in sensitivity for the several amines had begun to appear. The animals had recovered fully from operation, were in good health, and in receipt of full substitution therapy (Table 5), when the responses of the heart rate to these amines were re-examined.

The effect of adrenalectomy after sympathectomy. Reference to Fig. 6 reveals that from the 20th week after sympathectomy, the increase in sensitivity in the responses of the heart rate to both adrenaline and noradrenaline, which resulted from sympathectomy, may be expected to decline. Further, Fig. 8 shows that whereas the increased sensitivity of the responses of the heart rate to tyramine is well maintained for many weeks, the changes produced in the sensitivity of these responses to β-phenylethylamine and amphetamine are of a more evanescent character.

Bitches nos. 2 and 3 were adrenalectomized during the period of decline from the maximum of the changes produced by sympathectomy in the responses of the heart rate to these several sympathomimetic amines. The responses of the heart rate were re-examined 2–7 weeks, and 3 months after adrenalectomy. The results are summarized in Table 4 and are illustrated in Fig. 9.

Bitch 2 was examined in the 3rd–7th weeks after adrenalectomy, 28–32 weeks after sympathectomy. The responses of the heart rate to the several sympathomimetic amines recorded during this period after adrenalectomy may be compared, in Table 4, with those observed 20–24 weeks after sympathectomy, before adrenalectomy. The slight decline in sensitivity to all these amines other than adrenaline, recorded in the period 3–7 weeks after adrenalectomy, should most probably be related to the lengthened period after sympathectomy. The absence of a decrease in sensitivity to adrenaline might be related to the onset changes due to adrenalectomy. Bitch 3 was examined 2 weeks after adrenalectomy, 21 weeks after sympathectomy. From Fig. 9 and Table 4 it is seen that there was a decline in sensitivity of the heart-rate responses to all these amines, when the results are compared with those recorded 10–16 weeks after sympathectomy. This decline might have been expected to occur as a result of an increase in the time interval after sympathectomy (Figs. 6 and 8). This same animal was re-examined 10–15 weeks after adrenalectomy, and 29–34 weeks after sympathectomy (Table 4 and Fig. 9). The decrease in the sensitivity of the heart-rate responses to these amines, which might have been expected to result from the lengthened time interval after sympathectomy, was evident only for the responses of the heart rate to amphetamine and to β-phenylethylamine. The tyramine responses were well maintained. Potentiation of the responses of the heart rate to both adrenaline and noradrenaline had undoubtedly occurred.

Four examinations were made of the responses of the heart rate to adrenaline, noradrenaline and tyramine, in bitch 2, 17 weeks after adrenalectomy, and

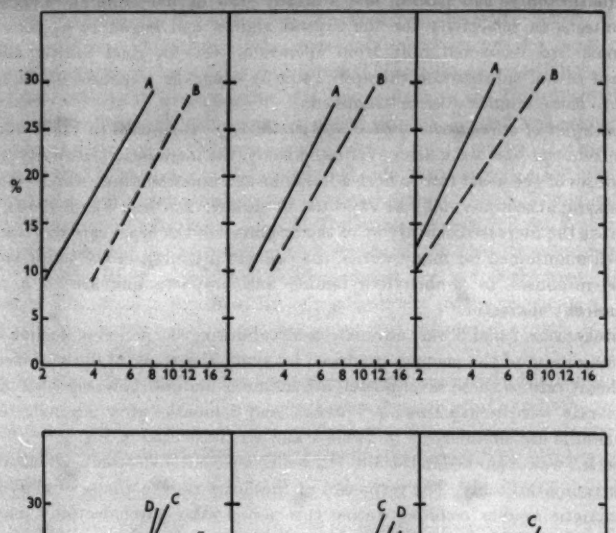

Fig. 9. Relation between percentage increase in heart rate and dose of amine hydrochloride, for bitch 3, after sympathectomy, and at intervals after subsequent adrenalectomy. Abscissae and ordinates as in Fig. 5. *A*, L-adrenaline; *B*, DL-noradrenaline; *C*, tyramine; *D*, β-phenylethylamine; *E*, amphetamine. Left, 10–16 weeks after sympathectomy; centre 2 weeks after adrenalectomy, 21 weeks after sympathectomy; right, 10–15 weeks after adrenalectomy, 29–34 weeks after sympathectomy.

41 weeks after sympathectomy. The observations recorded confirm the fact that potentiation of the responses of the heart rate to both adrenaline and

noradrenaline occurs in sympathectomized animals, some months after adrenalectomy. The increase in sensitivity of the responses of the heart rate toward tyramine was again well maintained. This feature characterized the responses of the heart rate to tyramine at a late stage after sympathectomy, and cannot be related to the alterations produced by a subsequent adrenalectomy on the evidence here presented.

The effect of adrenalectomy, followed by sympathectomy. Removal of the sympathetic chains, from the stellate ganglia into the pelvis, must have produced a pre-ganglionic denervation of the suprarenal glands. Such a denervation might be expected to influence the sensitivity of these glands to sympathomimetic amines (Lockett, 1950*b*). It was therefore possible that some of the alterations, which followed sympathectomy, in the responses of the heart rate to sympathomimetic amines, should in fact originate from coincident changes in the suprarenal glands. To test this hypothesis, bitch 4 was first adrenalectomized, and then sympathectomized; the changes in the responses of the heart rate to sympathomimetic amines which resulted from these two operative procedures were observed, and compared with the results recorded in Part II of this paper.

The curves relating percentage increase in heart rate to weight of amine hydrochloride, injected intravenously, are shown for the several amines, for bitch 4 before operation, in Table 2 and Fig. 10. This animal before adrenalectomy had proved highly insensitive to sympathomimetic amines. Six to ten weeks after adrenalectomy, there was some increase in the responses of the heart rate to both adrenaline and noradrenaline, and there was a much greater increase in the responses of the heart rate to tyramine, β-phenylethylamine and amphetamine (Fig. 10).

At an early stage (5th week) after sympathectomy of this adrenalectomized bitch, the resultant changes in the responses of the heart rate to the several sympathomimetic amines differed, in some respects, from those of normal dogs sympathectomized, and examined at an early stage post-operatively (Table 4). As after sympathectomy in normal animals, the responses of the heart rate to adrenaline were increased more than to noradrenaline, and the responses to amphetamine were reduced by the sympathectomy of this adrenalectomized animal. In contrast to the effect of sympathectomy on the responses of the heart rate in normal animals, in bitch 4, adrenalectomized before sympathectomy, the early potentiation of the response of the heart rate to β-phenylethylamine was replaced by reduced sensitivity, and the responses of the heart rate to tyramine were decreased. Unfortunately, the sleep reflex of this animal was destroyed by rebuilding operations; further observations were not made.

The observations made on these dogs, adrenalectomized before and after sympathectomy, indicate that a part of the explanation of the changes occurring in the responses of the heart rate to sympathomimetic amines after sympa-

thectomy is to be sought in the functional alterations in the suprarenal glands which takes place as a result of pre-ganglionic denervation.

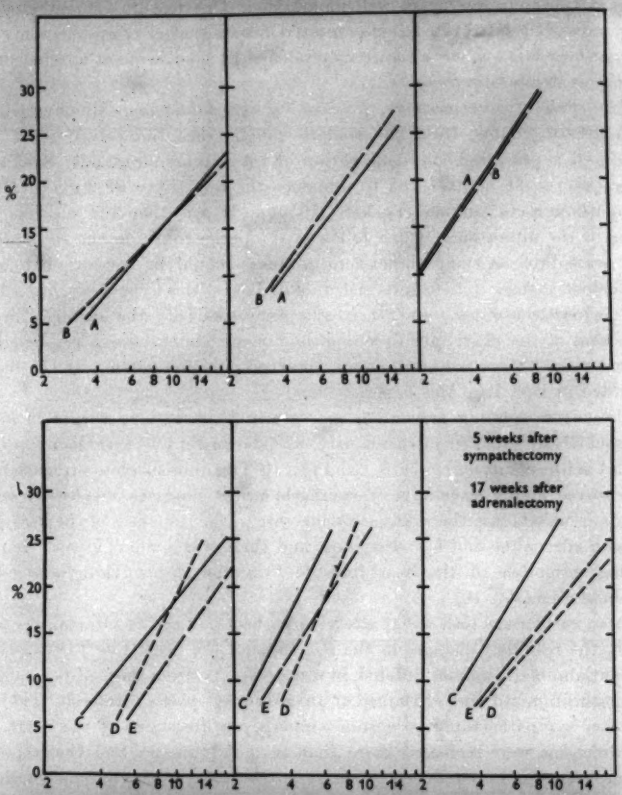

Fig. 10. Bitch 4. Relation between percentage increase in heart rate and dose of amine hydrochloride, before (left) and 6–10 weeks after (centre) adrenalectomy, and after both adrenalectomy and sympathectomy (right). Abscissae and ordinates as in Fig. 5. *A*, adrenaline; *B*, noradrenaline; *C*, tyramine; *D*, amphetamine; *E*, β-phenylethylamine.

The responses of the systolic blood pressure

In four bitches, in which the responses of the heart rate to sympathomimetic amines were being examined (Table 2, nos. 1–3 and 5), mean curves relating the log dose of amine hydrochloride to rise of systolic blood pressure in mm. Hg were determined for the several amines, injected intravenously. The

TABLE 6. The responses of the systolic blood pressure to sympathomimetic amines before and after sympathectomy

Dog	Amine hydrochloride	Mean dose-response curve: μg. amine hydrochloride producing rise in B.P. 20 mm. Hg *a*	50 mm. Hg *b*	Slope of curve: Ratio *b*/*a*	No. of determinations	Standard deviation of response	Weeks after sympathectomy
3	DL-Noradrenaline	3·35	5·8	1·73	10	2·6–2·8	Normal
	L-Adrenaline	3·6	6·15	1·62	10	2·2–2·5	
	Tyramine	280	430	1·54	6	3·0–2·6	
	Amphetamine	365	520	1·42	6	2·9–1·6	
	β-Phenylethylamine	425	740	1·74	6	3·4–3·0	
	DL-Noradrenaline	1·05	2·15	2·05	6	2·4–2·2	Third
	L-Adrenaline	1·73	3·0	1·73	6	2·7–2·2	
	β-Phenylethylamine	124	300	2·42	6	3·4–2·0	
	Tyramine	165	620	3·76	6	2·6–2·3	
	Amphetamine	440	940	2·14	6	2·5–2·1	
	DL-Noradrenaline	1·06	1·92	1·81	4	3·1–2·4	Fifth
	L-Adrenaline	1·95	3·3	1·69	4	2·5–1·7	
	β-Phenylethylamine	105	310	1·77	4	2·5–2·2	
	Tyramine	175	860	4·91	4	2·1–1·3	
	Amphetamine	430	800	1·86	3	2·0–1·2	
1	L-Adrenaline	3·6	8·0	2·22	10	2·2–1·6	Normal
	DL-Noradrenaline	3·75	8·0	2·13	10	3·2–1·5	
	Tyramine	360	700	1·94	8	2·4–1·9	
	Amphetamine	350	750	2·14	5	3·7–1·3	
	β-Phenylethylamine	480	1130	2·35	8	3·4–1·8	
	DL-Noradrenaline	1·65	2·3	1·39	6	3·0–1·9	Fourth
	L-Adrenaline	2·05	3·55	1·73	6	2·1–1·6	
	β-Phenylethylamine	143	430	3·01	4	1·6–1·0	
	Tyramine	205	1320	6·44	4	1·9–1·3	
	Amphetamine	425	720	1·69	4	2·9–1·2	
	DL-Noradrenaline	1·55	2·8	1·81	4	2·0–1·1	Sixth
	L-Adrenaline	2·2	3·75	1·70	4	1·8–1·7	
	β-Phenylethylamine	175	440	2·51	4	2·6–1·5	
	Tyramine	285	1240	4·35	4	4·1–1·9	
	Amphetamine	440	860	1·95	4	2·3–1·3	
2	L-Adrenaline	3·35	7·4	2·21	10	3·3–2·3	Normal
	DL-Noradrenaline	3·95	8·2	2·08	10	3·0–2·1	
	Tyramine	310	500	1·61	10	3·4–2·6	
	Amphetamine	345	540	1·57	5	3·2–2·0	
	β-Phenylethylamine	495	740	2·49	10	3·1–2·4	
	DL-Noradrenaline	1·2	2·42	2·02	6	2·9–2·2	Fifth
	L-Adrenaline	1·45	3·7	2·55	6	2·6–1·3	
	β-Phenylethylamine	208	330	1·62	6	3·6–2·3	
	Tyramine	230	700	3·04	6	2·9–2·0	
	Amphetamine	440	1000	2·27	3	2·3–1·2	
	DL-Noradrenaline	1·65	3·3	2·00	6	2·7–1·2	Eighth
	L-Adrenaline	1·8	4·4	2·44	6	3·5–1·8	
	β-Phenylethylamine	210	500	2·38	6	2·1–1·6	
	Tyramine	250	640	2·56	6	2·4–2·2	
	Amphetamine	325	1020	3·14	4	3·2–3·1	
5	L-Adrenaline	4·5	6·5	1·44	7	1·9–1·9	Normal
	DL-Noradrenaline	5·0	7·3	1·46	7	1·8–1·1	
	Tyramine	505	650	1·29	6	2·6–1·7	
	Amphetamine	480	705	1·47	3	3·3–2·8	
	β-Phenylethylamine	580	960	1·71	6	3·5–3·2	
	DL-Noradrenaline	1·43	2·05	1·43	7	3·4–2·4	Fourth
	L-Adrenaline	2·25	4·7	2·09	7	4·5–3·1	(from just
	β-Phenylethylamine	255	720	2·82	6	5·0–2·5	below
	Tyramine	505	1180	2·33	6	4·4–1·8	stellates)
	DL-Noradrenaline	1·10	2·63	2·39	6	3·6–1·6	Seventh
	L-Adrenaline	2·2	5·65	2·57	6	2·9–1·7	
	β-Phenylethylamine	300	640	2·13	6	3·3–2·0	
	Tyramine	480	880	1·83	6	1·6–1·2	
	Amphetamine	580	1480	2·55	3	4·2–2·2	

sympathetic chains of three of these animals were removed from just above the stellate ganglia down to the pelvis. In the fourth animal, no. 5, sympathectomy

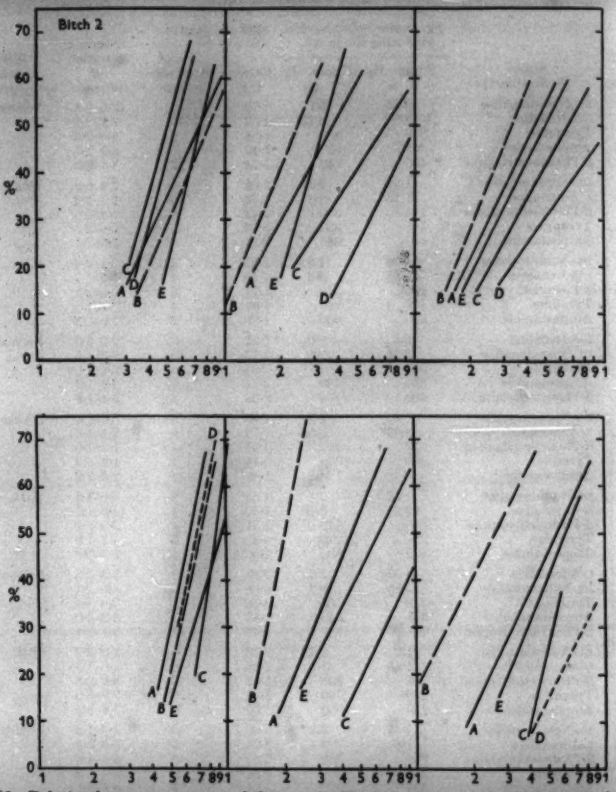

Fig. 11. Relation between responses of the systolic blood pressure and weight of amine hydrochloride, for two bitches, before and after sympathectomy. Ordinates: rise in systolic blood pressure. Between the first two sets of curves for each animal, the sympathetic chains were removed; in bitch 2 from just above the stellate ganglia, in bitch 5 from just below the stellate ganglia, downward into the pelvis. Left, before; centre, 5 weeks (bitch 2) and 4 weeks (bitch 5) after sympathectomy; right, 8 weeks (bitch 2) and 7 weeks (bitch 5) after sympathectomy. *A*, *B*, *C*, *D*, *E* as in Fig. 10.

extended from just below the stellate ganglia into the pelvis. The responses of the systolic blood pressure to the same amines were redetermined after operation. The results are summarized in Table 6.

From the first three dogs listed in Table 6 the stellate ganglia were removed with the thoracic chains, and the hearts were therefore completely sympathectomized. Comparison of the weights of amines used for heart rate and systolic blood-pressure determinations (Tables 2, 4 and 6), shows that the latter were less than one-half the former, and should have produced an increase in heart rate never exceeding 7%. In these three animals (Table 6), the increase in sensitivity of the systolic blood-pressure responses produced by sympathectomy was greater for noradrenaline than for adrenaline. There was a marked increase in sensitivity to β-phenylethylamine, which, in all three animals, was lost by the 8th week from the mid-stage operation (Fig. 11). Sensitivity of the blood-pressure responses to tyramine was decreased by sympathectomy; a similar but more marked decrease was observed for amphetamine.

The last dog listed in Table 6 underwent removal of the sympathetic chains from just below the stellate ganglia downward into the pelvis. This procedure was adopted to minimize the distortion of the observations on the systolic blood pressure by coincident changes in the sensitivity of the heart-rate responses to sympathomimetic amines. The alterations in the responses of the systolic blood pressure produced by this procedure for noradrenaline, adrenaline, tyramine and amphetamine, resemble those previously recorded for bitches 1, 2 and 3 (Table 6), but the differences between the individual amines appear slightly accentuated. The early potentiation of the β-phenylethylamine response is much less evident. These observations are illustrated in Fig. 11.

DISCUSSION

Konzett & Barcroft (1949) found that when noradrenaline and adrenaline were infused intravenously into normal human subjects in amounts which produced an approximately similar rise of blood pressure, only noradrenaline produced bradycardia; adrenaline produced tachycardia. In the experiments here described, performed on sleeping bitches, larger weights of atropine have been found necessary to convert the heart-rate responses to single intravenous injections of noradrenaline and β-phenylethylamine from bradycardia to tachycardia than were required either for adrenaline or for tyramine. It therefore appears probable that the bradycardia due to noradrenaline, described by Konzett & Barcroft, was reflex in origin, effected through the vagus.

Mean curves, relating the percentage increase in heart rate to the log dose of amine hydrochloride, injected intravenously into fully atropinized sleeping bitches, have been found to remain constant over a period of months; the relative slopes and positions of these curves for the several amines differed widely however, as between nine individuals, irrespective of the weights of the animals. These results are held to justify the conclusion that such mean curves constituted 'characteristics' for the individuals, and that, at least so far as the

effects of sympathomimetic amines on the heart rates of fully atropinized bitches are concerned, there may exist receptor patterns characteristic of the individual. The alternative view that such variation may be due to differences in receptor patterns, may well be offered. Discussion of these two possibilities is delayed until evidence concerning the effects of anaesthesia, and of dibenamine, on the responses of the heart rate and systolic blood pressure to sympathomimetic amines, and the measurements of the relative changes in the positions and slopes of the log dose-response curves for the several amines in individual animals from day to day has been presented.

Removal of the sympathetic chains from above the stellate ganglia downward into the pelvis must, presumably, have produced a post-ganglionic denervation of the heart and blood vessels other than those of the splanchnic area, and a pre-ganglionic denervation of the suprarenal glands and vessels of the splanchnic area. Whereas such a sympathectomy produced a greater increase in sensitivity of the heart rate to adrenaline than to noradrenaline, the reverse was true for the effect on the responses of the systolic blood pressure to these two amines. Bülbring & Burn (1949) have shown that post-ganglionic denervation of the nictitating membrane in cats results in a greater increase in sensitivity to noradrenaline than to adrenaline. The responses of the heart rate after sympathectomy differ from those of the nictitating membrane after post-ganglionic denervation. Burn (1938) showed abolition of the responses of the forelimb vessels to tyramine after post-ganglionic denervation, and demonstrated the differences between nictitating membrane and peripheral blood vessels in this respect. The changes produced in the responses of the heart rate to sympathomimetic amines by removal of the sympathetic chains are not unlike those produced in the nictitating membrane of the cat by pre-ganglionic denervation, in respect of the increased sensitivity to adrenaline, noradrenaline, β-phenylethylamine, and tyramine (Lockett, 1950*b*). The ganglia of Wrisberg, in the superficial cardiac plexi, have long been considered a part of the parasympathetic nervous system. Woollard (1926) investigated the intracardiac ganglia of dogs, and concluded that these ganglion cells were all parasympathetic. Billingsley & Ranson (1918) described a constant communication between the superior cervical ganglion and the ganglion nodosum of the vagus, in cats; pre-ganglionic section of sympathetic fibres, which had a synapse in the superior cervical ganglion, and passed to the heart by the vagus, could have resulted from removal of the stellate ganglia. However, a strict parallel between the responses of tissues so fundamentally different as heart and nictitating membrane cannot be expected.

Observations on the actions of these four amines on the denervated suprarenal gland are in progress, but are, as yet, incomplete. The partially denervated gland has been shown to have an increased sensitivity to nerve stimulation (Simeone, 1938); the denervated gland may be altered not only in sensitivity

to amines but also in the nature of its hormone output as the result of stimuli. It cannot, therefore, be inferred that the receptors for the effect of sympathomimetic amines on the heart rate are fundamentally different from those of the vascular bed, until more information is available relating to the function of the denervated suprarenal gland.

SUMMARY

1. The methods required to obtain a minimum day-to-day variation in the responses to the same doses of sympathomimetic amines, and to yield steady base-lines in long experiments lasting up to 4 hr. on unanaesthetized, fully atropinized bitches, are described.

2. Eighty per cent of conscious dogs tolerated subcutaneous atropine sulphate in a dose of 0·8 mg./kg. The remainder tolerated 0·6 mg./kg. Undesirable side effects encountered in ten dogs after 1 mg./kg. of atropine sulphate are described.

3. The mean curves of day-to-day observations, relating percentage increase in heart rate to log dose of amine salt in fully atropinized sleeping bitches were determined for eight animals. The amines tested were L-adrenaline, DL-noradrenaline, tyramine, β-phenylethylamine and amphetamine. The differences between the relative positions and slopes of the mean curves for the several amines differed so widely from one dog to another, irrespective of their weight, as to preclude the use of a control group of normal bitches for comparison with those that had undergone sympathectomy or adrenalectomy. The constancy of the mean curves in any given individual was, however, demonstrated for periods of months.

4. Removal of the sympathetic chains from just above the stellate ganglia downward into the pelvis affected the responses of the heart to sympathomimetic amines in atropinized sleeping bitches differently from the responses of the systolic blood pressure. The heart-rate responses showed a greater increase in sensitivity to adrenaline than to noradrenaline. Responses to β-phenylethylamine, and to tyramine, were increased; those to amphetamine were decreased. The responses of the systolic blood pressure were increased more to noradrenaline than to adrenaline: the responses to β-phenylethylamine were increased, but those to tyramine and to amphetamine were decreased.

5. Adrenalectomy in two sympathectomized dogs resulted in a delayed increase in the responses of the heart rate to adrenaline and noradrenaline, no alteration in its sensitivity to tyramine, and a reduction in its responses to β-phenylethylamine; the latter should probably be related to an increasing time interval after sympathectomy. Adrenalectomy in one animal resulted in an increase in the responses of the heart rate to all these amines. Subsequent sympathectomy produced a greater increase in the responses of the heart rate to adrenaline than to noradrenaline, and a reduction of the responses to

amphetamine, as in animals sympathectomized without previous adrenalectomy. The increased sensitivity of the heart rate to β-phenylethylamine seen 6–8 weeks after the sympathectomy of normal animals was replaced in that adrenalectomized before sympathectomy, by a reduction in the responses of the heart rate to β-phenylethylamine.

A part of the expense of this work was defrayed by a grant from the Medical Research Council. Prof. E. C. Kendall very kindly sent the extract of suprarenal gland and Dr M. L. Tainter the noradrenaline used in these experiments. I wish to thank my two technicians, Joan Nunn and Phyllis Harris, for their assistance at operations and dressings, and in the maintenance of the dogs.

REFERENCES

Billingsley, P. R. & Ranson, S. W. (1918). *J. comp. Neurol.* **29**, 375.
Bülbring, E. & Burn, J. H. (1938). *J. Physiol.* **91**, 459.
Bülbring, E. & Burn, J. H. (1949). *Nature, Lond.*, **163**, 363.
Burn, J. H. (1938). *Physiol. Rev.* **8**, 137.
Burrett, J. B. (1940). *Amer. J. Physiol.* **131**, 415.
Konzett, H. & Barcroft, H. (1949). *Lancet*, i, 147.
Leersum, E. C. V. (1911). *Pflüg. Arch. ges. Physiol.* **142**, 377.
Lockett, M. F. (1949*a*). *Brit. J. Pharmacol.* **4**, 111.
Lockett, M. F. (1949*b*). *J. Physiol.* (in the Press).
Lockett, M. F. (1949*c*). *J. Physiol.* **109**, 146.
Lockett, M. F. (1950*a*, *b*). In preparation.
Selye, H. (1936). *Brit. J. exp. Path.* **17**, 234.
Simeone, F. A. (1938). *Amer. J. Physiol.* **122**, 186.
Verney, E. B. & Vogt, M. (1938). *Quart. J. exp. Physiol.* **28**, 253.
Woollard, H. H. (1926). *J. Anat., Lond.*, **60**, 345.

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THE END-PLATE POTENTIALS OF THE RAT DIAPHRAGM PREPARATION

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Preparations of mammalian muscle from which end-plate potentials may be obtained have been described by Eccles & O'Connor (1939) and by Brown & Burns (1949). In both cases, however, the circulation to the muscle was intact and drugs and other modifying agents were injected into it. For some purposes, and particularly for the investigation of ionic effects, it would be useful to have an isolated mammalian preparation which would give reasonably large end-plate potentials. We therefore investigated the suitability of the isolated phrenic-diaphragm preparation of the rat for this purpose.

METHODS

The preparation was made by the technique described by Bülbring (1946), but the strip of muscle was usually cut from the right side of the diaphragm as this contains more muscle and less tendon than the more usual left side preparation, though the nerve is slightly more difficult to dissect.

The apparatus consisted of a Perspex bath in which the muscle could be fixed horizontally, the tendon being attached either to a lever system or to a crystal pick-up as a tension recorder. An outer water-bath maintained a constant temperature.

The recording electrodes were of silver wire fused into glass capillary tubing and were about 80–100 μ in diameter at the tip. They were cleaned by honing before each experiment. These electrodes were mounted on the outer bath and two screw adjustments were provided giving vertical and horizontal motions, so that records could be taken every $\frac{1}{2}$ mm. along the muscle with the two electrodes a fixed distance apart, their position being recorded on a millimetre scale. In certain experiments only one such travelling electrode was employed, the indifferent electrode being a silver wire immersed in the fluid in the bath. A lateral traverse of the muscle was obtained by a sliding movement of the inner Perspex bath.

The phrenic nerve was pulled through two silver loop electrodes and was stimulated by an electronic square wave generator. The stimulus was usually 0·5–1·0 V. with a duration of 0·1 msec., which was found to be supramaximal for nerve stimulation. The action potentials of the muscle were fed into a capacity-coupled balanced amplifier followed by a compressor stage, and were displayed on a double-beam cathode ray tube. The overall time-constant of the amplifier was 75 msec.

In the experiments on summation of the end-plate potentials, the output of the square-wave stimulator was passed through the primary of an air-cored induction coil, while the secondary was

connected to the stimulating electrodes. The time between the two stimulating voltage spikes could then be varied by altering the duration of the square-wave.

In all cases the muscle was suspended in Krebs solution through which a mixture of 95% oxygen and 5% CO_2 was bubbled. The entire muscle and nerve were beneath the surface of the solution.

RESULTS

Longitudinal traverses of the preparation in normal Krebs solution with the electrodes about 2 mm. apart showed action potentials, diphasic or multiphasic, at all points as would be expected. The peak voltages of the spikes varied considerably during a traverse, presumably mainly due to summation effects, but the usual value was about 5–10 mV.

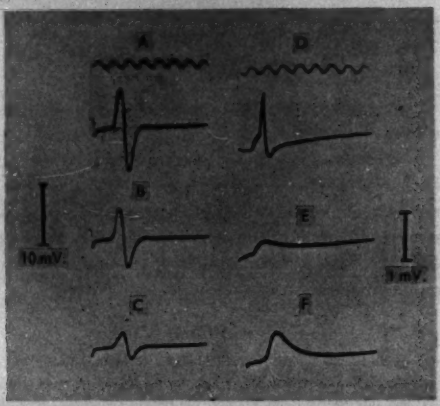

Fig. 1. Muscle action potentials of isolated rat diaphragm showing successive stages after the addition of tubocurarine (1 μg./c.c.) between frames A and B. Between frames C and D the amplification was increased about ten times and between frames E and F the electrodes were moved slightly to find the optimum end-plate potential. Recording electrodes 2·5 mm. apart. Time, 1000 cyc./sec.

When curare was added to the bath to give a concentration of 1–2 μg./c.c. the muscle action potentials became progressively smaller and disappeared. At this stage, if the electrodes were moved along the muscle it was possible to record at certain points a monophasic potential, more prolonged than the muscle action-potential spike and about 0·3–0·6 mV. in magnitude. This is shown in Fig. 1, the duration of the spike potential being 1 msec., whereas that of the monophasic potential is at least 4 msec. This latter potential was not conducted as it disappeared on moving the active electrode a few mm.

Fig. 2, taken from a different experiment with unipolar recording, shows that on removing the curare block gradually by progressive dilution and replace-

ment of the solution, a muscle action-potential spike grew up on the monophasic potential. The addition of eserine (0·2 μg./c.c.) to the curarized muscle caused a similar effect.

By moving the electrodes along the diaphragm from tendon to ribs in parallel traverses after curarization, it was found that these low voltage monophasic potentials were confined to certain regions of the preparation, though

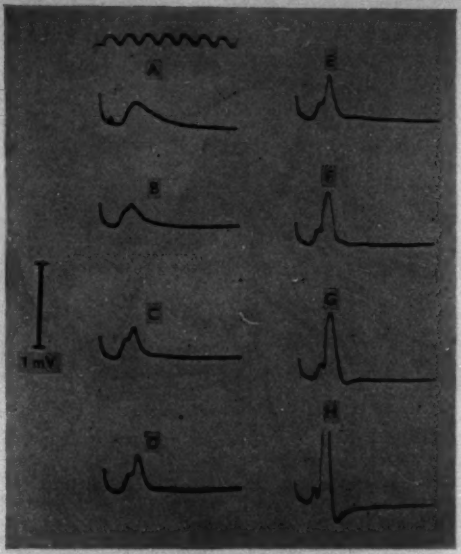

Fig. 2. Frame A shows the end-plate potential of the curarized rat-diaphragm. Between frames A and B the bath fluid was partially replaced to remove the curare block, and frames B–H show successive stages in the growth of the muscle action-potential spike. Unipolar recording. Time, 1000 cyc./sec.

there were slight variations from muscle to muscle. Fig. 3 gives a diagram of the upper surface of the preparation from the right half of the rat diaphragm showing the area over which these potentials are most likely to be found. It forms a strip running across the muscle, approximately midway between tendon and ribs, and situated a few mm. on the rib side of the point of entry of the phrenic nerve. The potentials are usually most readily obtained towards the medial side of the preparation and are difficult to pick up near the lateral edge as the diagram indicates. The same description also holds good for the left-side preparation which, of course, is in the form of a mirror-image of the present diagram.

Fig. 4 shows a small portion of a typical longitudinal traverse of the curarized muscle with the electrodes 3 mm. apart and moved 0·5 mm. between each frame. As the end-plate region was approached by the leading electrode, the monophasic potential appeared and increased to a maximum within 1·5 mm., showing that it was not conducted. Similar results were obtained using a single exploring electrode and an indifferent electrode.

Fig. 3. Diagram of the upper (thoracic) surface of a preparation from the right half of the rat diaphragm. The area over which end-plate potentials are most likely to be obtained is shown cross-hatched.

When two stimuli were applied to the nerve at increasing intervals, two monophasic potentials appeared when the interval was greater than about 0·8 msec. With deep curarization, though summation was present and the second potential was greater than the first, no conducted action potential appeared as a result. With light curarization, however, the second potential became great enough to set up a conducted spike, as shown in Fig. 5.

It was later found that the most rapid and convenient method of locating the end-plate region was to add a very small amount of tubocurarine to the bath to

give a concentration of 0·5 μg./c.c. or less. At the usual frequency of stimulation of 12 per min. a large conducted muscle action potential remained after this dose. If now, however, the frequency of nerve stimulation was increased

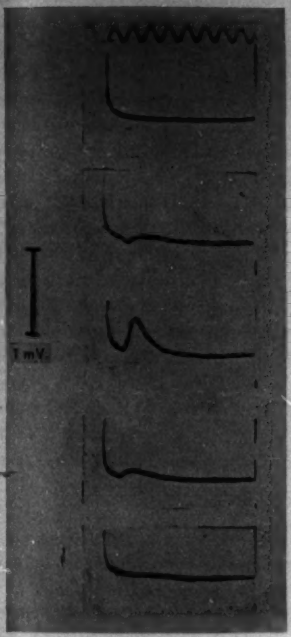

Fig. 4.

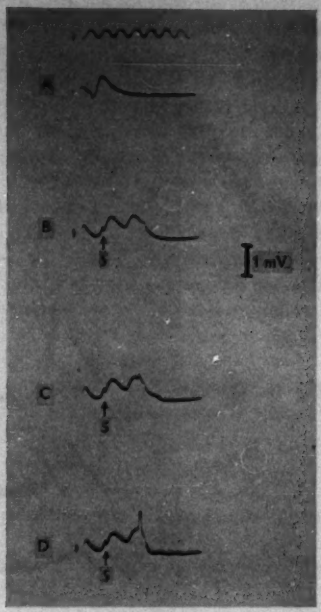

Fig. 5.

Fig. 4. Successive frames from part of a longitudinal traverse of the curarized rat-diaphragm preparation. Recording electrodes 3 mm. apart and moved 0·5 mm. towards the ribs between each frame. Time, 1000 cyc./sec.

Fig. 5. Potentials from curarized rat-diaphragm preparation when two stimuli were applied to the phrenic nerve. Frame A, single end-plate potential when stimuli were 0·7 msec. apart. Frames B, C and D show successive stages of summation of two end-plate potentials to set up a spike action potential when the interval between the stimuli was 1·6 msec. *S*, stimulus artefact of second stimulus to nerve. Time, 1000 cyc./sec. Unipolar recording.

to 100 per sec., the conducted action potential rapidly disappeared and with the electrode over the end-plate region a pure end-plate potential was obtained. As at this frequency a standing image was present on the oscilloscope it was easy to find the end-plate region by rapidly traversing the muscle until the end-plate potential appeared. Fig. 6 shows that by varying the frequency of

stimulation all stages between a muscle action potential and an end-plate potential could be obtained. With continued stimulation at 100 per sec. the end-plate potential remained constant for considerable periods up to 1½ hr. or more.

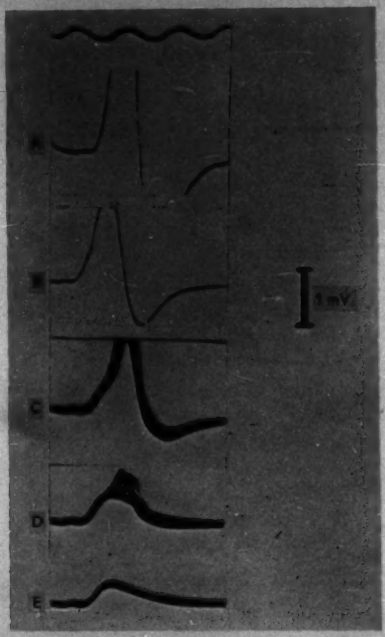

Fig. 6. Rat diaphragm preparation lightly curarized (0·5 μg. tubocurarine/c.c.). Frame A, nerve stimulated at a frequency of 12 per min. Frame B, stimulated at 2 per sec. Frame C, at 10 per sec. Frame D, at 50 per sec. Frame E, at 100 per sec. Time, 1000 cyc./sec. Frames C, D and E show several sweeps superimposed.

DISCUSSION

There seems little doubt that the monophasic potentials obtained were true end-plate potentials as they satisfied the following criteria:

(1) They were observed after partial curarization.

(2) They were confined to definite locations on the muscle.

(3) They were not conducted.

(4) On removal of the curare block a conducted muscle action potential spike appeared on the broader low voltage potential.

(5) On applying two stimuli to the nerve at an appropriate interval, summation of the potentials occurred.

There would, therefore, appear to be a concentration of end-plates in the rat diaphragm along a strip about mid-way between the central tendon and the ribs. This is rather surprising in view of the statement by Brown, Bülbring & Burns (1948) that the fibres of this preparation are usually only about 5 mm. in length.

The observation that during very light curarization progressive increase in the frequency of nerve stimulation first abolishes the action potential and then, with still higher frequencies, depresses the end-plate potential itself, obviously requires further investigation. Presumably there is a critical frequency at which the end-plate potential is depressed below the threshold required for triggering the muscle action potential. It is possible that end-plate potentials may be obtainable by this method in uncurarized muscle, but we were unable to test this satisfactorily owing to the limited frequency range of our apparatus. This phenomenon may perhaps be concerned in the mechanism of Wedensky inhibition as was suggested by Feng (1940, 1941), following somewhat similar experiments on amphibian preparations. In Feng's experiments, however, the volley of nerve impulses was continued only for some 100 msec., but within that period he found that the amphibian end-plate potentials were able to follow the stimulus frequency up to the limit set by the refractory period of the nerve itself.

The fact that no change in the end-plate potential was apparent over long periods at stimulus frequencies up to 100 per sec. was unexpected in view of the usual theories of neuromuscular 'fatigue'.

SUMMARY

1. A method of recording end-plate potentials from the isolated rat phrenic-diaphragm preparation is described.

2. End-plate potentials can be most easily observed in a region midway between the central tendon and the ribs.

3. Preliminary experiments on the effect of frequency of nerve stimulation on the end-plate potentials of this preparation are briefly discussed.

REFERENCES

Brown, G. L., Bülbring, E. & Burns, B. D. (1948). *J. Physiol.* **107**, 115.
Brown, G. L. & Burns, B. D. (1949). *J. Physiol.* **108**, 54*P*.
Bülbring, E. (1946). *Brit. J. Pharmacol.* **1**, 38.
Eccles, J. C. & O'Connor, W. J. (1939). *J. Physiol.* **97**, 44.
Feng, T. P. (1940). *Chin. J. Physiol.* **15**, 367.
Feng, T. P. (1941). *Biol. Symp.* **3**, 121.

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SOME PROPERTIES OF THE CAT'S ISOLATED CEREBRAL CORTEX

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This paper is concerned with the properties of the cat's cerebral cortex, treated as an isolated organ. The experiments have all been performed upon a layer composed of cortical grey matter with some of the underlying cortico-cortical fibres; this slab of tissue has been isolated from all connexion with either the central nervous system or the adjacent cortex, its blood supply being the only connexion with the rest of the animal.

An attempt to determine the properties of isolated cortical tissue scarcely needs justification. Although the electrical activity seen in a preparation of this sort is, no doubt, far removed from the behaviour of the intact cortex, the properties of the isolated cortex, despite its diverse cell types and unknown intercellular connexions seem, in the present state of knowledge, to make an easier subject for research than does the behaviour of the intact brain. A short summary of the preliminary work with the preparation has already been reported (Burns, 1949).

In reporting experiments carried out with a preparation of one type, it is easy to give the impression that the results obtained are applicable to all normal cortical tissue. I think it is important, therefore, to stress at the outset, the extremely limited nature of these experiments, all of which have been made on one small area of the cat's brain, using only one anaesthetic.

METHOD

Preparation of the animal. All the cats used for these experiments received an intravenous injection of between 6 and 8 c.c./kg. of 1% chloralose while under initial ether anaesthesia. Adult animals weighing between 2 and 3·5 kg. were used. A tracheal cannula was inserted and loops of thread passed round both carotid arteries, in order that these might be easily found and clamped at any later stage of the operation. The cat was then turned with its ventral side on the operating table and the head was clamped in a Tschermak holder so that the vertex was some 20 cm. above the table.

A skin incision was made in the mid-line, the temporal muscle was removed, and the skull was opened widely over the parietal and temporal surfaces of the dura-mater. Bleeding from the bone was reduced by clamping the appropriate common carotid, and, since finding that the clamping

of one carotid made no apparent difference to the electrical behaviour of the final preparation, I made a practice of leaving this clamp in place until the end of the operation. During this part of the operation the field was kept free from blood-clot and chips of bone by frequent irrigation of the dura with warm saline. The removal of the skull can usually be carried within 2–3 mm. of the mid-line without danger to the sagittal sinus or its tributaries. Once all bleeding from the cut edges of bone had stopped the dura was opened, cut free all round the margin of the exposed area and then lifted gently from the surface of the brain. (It was sometimes necessary to seal off with diathermy one or two small veins which run into the under surface of the dura.) Fig. 1 illustrates the remaining steps in the operation. A small piece of the cortex in the occipital region was removed (Fig. 1*a*) so that a knife could be easily inserted for cutting beneath the parietal surface of the brain. The precise location of the area removed from the posterior cortex depended largely upon the position of the venous drainage of that part of the cortex chosen for isolation. With diathermy it proved possible to remove the necessary portions of occipital cortex with little haemorrhage and without damage to the major veins. A knife, made from a strip of broken razor blade, was then

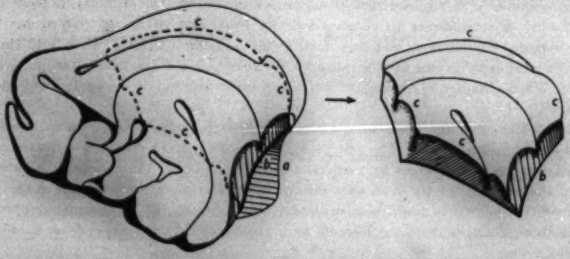

Fig. 1. A drawing from the left cerebral hemisphere of a cat in which the parietal cortex had been isolated from the rest of the nervous system. The slab of isolated cortical tissue is also shown as it would appear could it be removed from the cat. (See text.)

inserted through the base of the wound (Fig. 1*b*) and passed forward parallel to the surface of the parietal cortex. The knife was pulled gently across from lateral to medial side thus cutting all central connexions of the exposed cortex some 7 mm. beneath the surface. The final step, that of neurological isolation around the circumference of the chosen area, was performed with a piece of straight steel wire of about 1 mm. diameter bent to a right angle 7 mm. from its rounded tip. The bent wire was inserted in the plane of the cut made with the razor blade so as to pass beneath the cortical surface. By rotation of the shaft the tip was then brought up so that it appeared just beneath the pia mater. The tip of the wire was then worked carefully round the border (Fig. 1*c*) of the area of cortex to be isolated; during this process the tip was kept under close observation and with care it could always be made to slide harmlessly beneath the main pial vessels. The wire tip leaves in its wake a fine track, where it has separated the grey matter, which is made visible by slight haemorrhage. Occasionally the manœuvre with the wire caused a visible sub-pial haemorrhage and the preparation was discarded, but in general, little practice is necessary to prepare a neurologically isolated slab of cortical tissue without haemorrhage. The most accessible area for isolation included the median suprasylvian and median ectosylvian gyri and, in fact, the experiments reported in this paper have all been carried out using this area, or smaller areas within it.

Finally, the edges of the skin wound were tied with threads to a horizontal metal bar bent in to a circle and clamped just above the cat's head. In this way the skin flaps were converted into the walls of a reservoir which was filled with liquid paraffin.

Electrodes. Generally, silk wicks soaked in 0·9% NaCl solution were used. These were threaded into blunt-tipped stainless steel hypodermic needles, so that about 1 mm. of thread projected from the tip. This system has the advantage that it is easy to keep the thread moist by periodically injecting a drop of saline into the butt of the needle. The diameter of the silk in contact with the brain was about $\frac{1}{2}$ mm. These electrodes are of course polarizable, but mounted in this way the wicks have an effective capacity of some 2·0 μF. and when used in conjunction with cathode followers provide a time constant greatly in excess of the minimum required for these experiments. For convenience, these electrodes were made into banks of five or six; the needles were fixed in a row of holes drilled through a perspex block so that their tips emerged 1 or 2 mm. apart along a straight line.

The wicks can be used for stimulating as well as recording, but frequently it was more convenient to stimulate through constantan or platinum wires applied lightly to the surface of the brain.

Stimulation. For most purposes the stimuli were applied through surface electrodes arranged to touch the brain $\frac{1}{2}$–1 mm. apart and lying in a line at right angles to the line of recording electrodes. This arrangement is convenient because it minimizes stimulus artefact, but similar results have been obtained with unipolar surface negative stimulation. The stimulating leads were connected through a transformer to an electronically controlled source of rectangular waves of constant voltage. The duration and amplitude of the individual stimuli varied with the requirements of different experiments, but the range 0·1–0·9 msec. × 0–25 V. includes all stimuli which have been used. When stimulating through wicks, only a small fraction of the applied voltage reaches the brain.

Recording. The recording leads were connected through cathode followers to two five-stage push-pull amplification systems. The first three stages were battery operated and were connected to the last two mains fed stages by variable capacity couplings. The time constants used were usually of the order of 0·1 sec. and were always far greater than the duration of the potential disturbances studied. (It is useful to keep the time constants at the highest manageable value in order to restrict stimulus artefact.) Records were photographed upon paper from a double-beam cathode-ray tube.

Usually the amplifiers were each connected to two leads, a short distance apart on the surface of the cortex; for some purposes monopolar recording was used with an 'inert' lead in the cat's mouth as reference point, but this system is liable to record potential disturbances which are not located in the isolated area. All records from experiments in which the amplifier was connected to a single cortical lead show surface negativity as an upward deflexion. In all the bipolar records, when the lead nearest to the expected source of a potential disturbance became negative to the distal lead, there was an upward deflexion of the trace.

RESULTS

General properties of the isolated cortex

Neurological isolation of the cortex resulted in complete electrical inactivity. Records from two wicks placed 2 mm. apart upon the cortical surface showed no potential fluctuations, nor were any potential changes demonstrable between a single surface-electrode and earth. Fig. 2 shows records obtained from the same cortical surface points (*a*) before, and (*c*) after, isolation of the area containing those points. These methods of recording would, of course, only be expected to demonstrate the synchronized activities of large groups of cortical cells, and consequently the records only demonstrate the absence of massed spontaneous activity. However, no activity was recorded when a glass-pore electrode of 40 μ internal diameter at the tip was thrust into the grey matter

to a variety of depths. It appears, therefore, that under chloralose anaesthesia, neurological isolation of the parietal cortex results in complete electrical silence of the nerve cells and no spontaneous activity of these cells, synchronous or asynchronous, can be demonstrated.

Despite this inactivity, the isolated cells are not dead. A single stimulus applied through surface electrodes produced a local response in which the tissue beneath these electrodes became negative to the rest of the brain. This

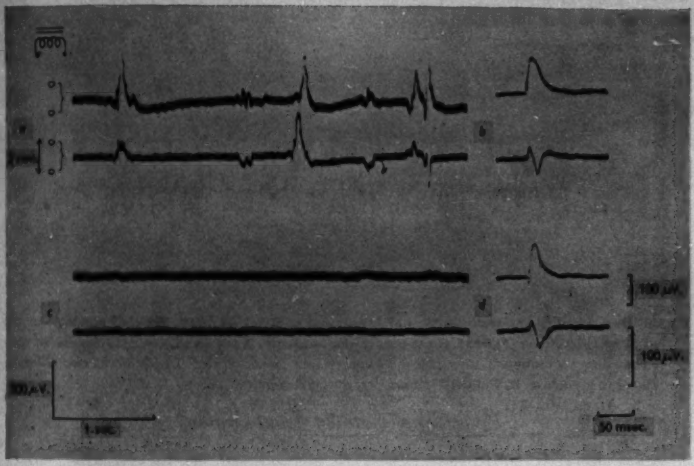

Fig. 2. Records obtained from the same points on the cortical surface before (*a* and *b*) and 70 min. after (*c* and *d*) isolation of the area beneath the electrodes. *a* = record of spontaneous activity; *b* = response to electrical stimulus; *c* = spontaneous activity after isolation; *d* = response to same stimulus after isolation. (Recording electrodes at 2, 4 and 6, 8 mm. distance from stimulating electrodes. Amplifier time constant = 200 msec.)

is the superficial local response described by Adrian (1936). These responses were not altered by the process of isolation: Fig. 2*b*, *d* show the responses of the same cortical points to weak stimuli of the same strength, both before and after the area containing these points had been isolated. Moreover, repetitive stimulation could produce in the isolated cortex a type of after-discharge known to occur in the intact cortex. Fig. 3 shows a part of the after-discharge caused by repeated stimulation at a frequency of 33/sec. These discharges resemble very closely those described by Adrian (1936), and consist in their simplest form of a series of potential waves spreading out from the point which has been stimulated.

These experiments with electrical stimulation certainly proved that the isolated area contained living nerve cells, but it seemed that such tests as these might be too crude to demonstrate damage resulting from the operation. An electrical stimulus applied through surface electrodes must excite many structures which, under normal circumstances, are never excited simultaneously. The fact that some sort of response was obtained from these tissues does not necessarily prove that the procedure of isolation had not disturbed their more subtle physiological activities; the absence of spontaneous activity might itself be a sign of injury.

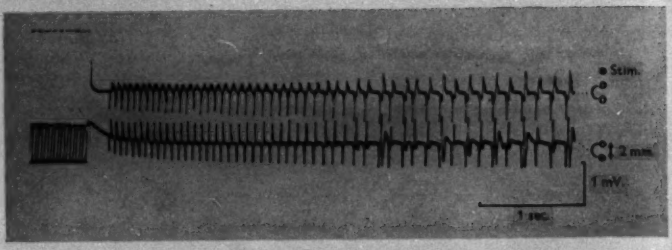

Fig. 3. Records of the after-discharge which followed stimulation of the isolated cortical surface at 33/sec. for 20 sec. The end of the period of stimulation is shown on the left; the record only shows the beginning of the after-discharge which lasted 8 sec. (Amplifiers, a.c. coupled. Recording leads at 2, 4 and 10, 12 mm. from the stimulated point.)

Properties of partially isolated cortex

It seemed probable that if an isolated slab of cortex was left attached to normal cortex by a bridge of undamaged tissue, the spontaneous rhythm of the undisturbed brain might enter the isolated portion by way of the bridge. The slab of isolated cortex was prepared in the usual way, except that separation around the circumference was incomplete; the cut made with the bent wire was restricted so as to leave a 'gateway' of normal cortex leading out into the uninjured parts of the hemisphere. The amount of manipulation of the brain—and consequently, the chances of injury to the isolated cells—were as great in this operation of partial isolation as they were in the preparation of the completely isolated specimen. It was found that provided the bridge of tissue connecting the normal and the isolated areas exceeded some 5 mm. in width (measured across the surface) the spontaneous activity of the normal brain would excite activity in the isolated portion.

Fig. 4 shows records of activity from a partially isolated area which was excited by spontaneous waves passing from normal brain across a bridge of undamaged cortex. The records of Fig. 4 show also that the spread of these waves within the isolated area was directly related to the width of the con-

necting bridge; a reduction of the gateway from 15 to 6 mm. restricted the spread of incoming waves; while closure of the gateway left the isolated area silent. The activity excited in these partially isolated preparations does not

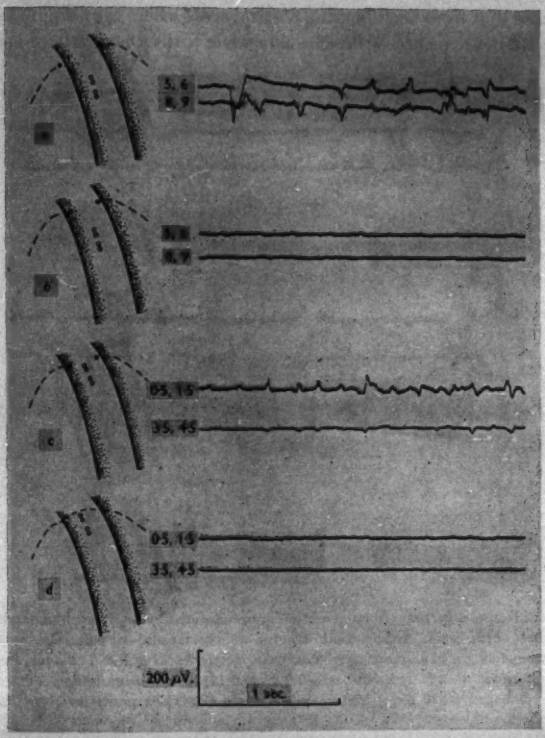

Fig. 4. Records from a partially isolated cortical area connected by a bridge of undisturbed cortex to the rest of the brain. On the left of each pair of records is a drawing representing the width of the bridge and the arrangement of electrodes. The distances of recording electrodes from the bridge or gate are given in mm. beside each trace. *a*, gate included two sulci and one gyrus. Effective cortical width 15 mm. *b* and *c*, gate includes gyrus only. Width 6 mm. *d*, gate closed and isolation of area complete.

always diminish with distance from the gate. Fig. 5 shows a similar preparation in which a gateway of only 5 mm. was exciting large waves of surface positivity which swept at 15 cm./sec. without attenuation to the borders of the isolated slab.

The experiments I have described point to the conclusion that the chloralosed parietal cortex has no power of spontaneous activity when isolated from neurological connexion with the rest of the nervous system. The spontaneous electrical rhythms demonstrable in this area before isolation must be either transmitted to it from neighbouring cortical areas where they originate, or may be in part a consequence of direct connexions with other parts of the central

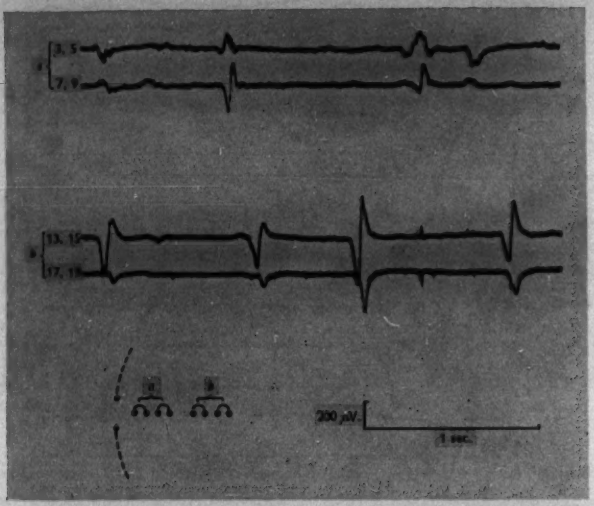

Fig. 5. Records from a partially isolated cortical area connected by a bridge of undisturbed cortex to the rest of the brain. Arrangement of 5 mm. wide bridge and recording electrodes is drawn at bottom left. *a*, simultaneous records from electrodes 3, 5 and 7, 9 mm. from bridge; *b*, simultaneous records from electrodes 13, 15 and 17, 19 mm. from bridge. (The cortex was visibly damaged at 19 mm.) The recorded waves of surface positivity were travelling at 15 cm./sec.

nervous system. That 'spontaneous activity' can be transmitted to the area in question from neighbouring cortical areas is, of course, demonstrated by the 'bridge' experiments described above. It proved possible, however, to isolate the area studied from all tangential connexions with the remainder of the cortex, while at the same time leaving intact some of the centrally running connexions from the cortex to the mid-brain. The tangential connexions were cut as usual to a depth of 7 mm. below the surface by inserting the bent wire through a number of points on the cortical surface, chosen so as not to damage the blood supply. But the cut made with the knife, parallel to the cortical

surface, was restricted so as to leave some of the central connexions intact. It was found that the area, when partially isolated in this way, still generated 'spontaneous' discharges; indeed, provided the anaesthetic was not too deep the partially isolated area was found to be peculiarly active. The reason for this

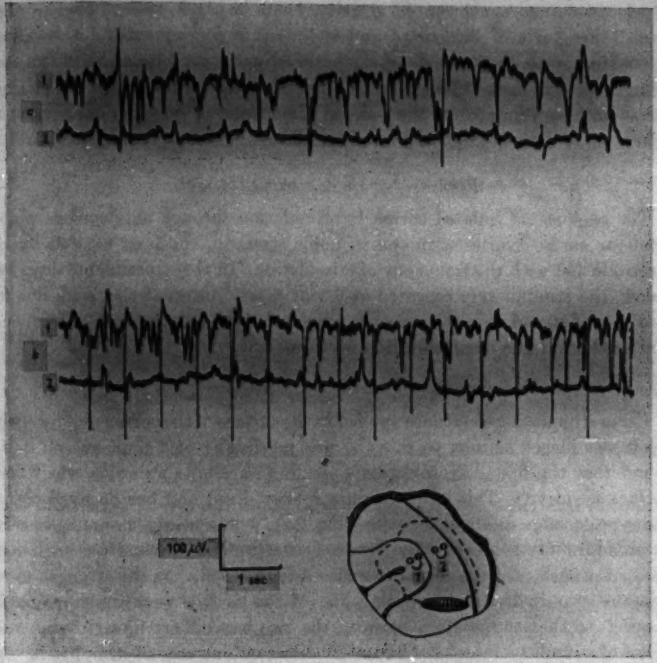

Fig. 6. Records from a partially isolated cortical area which was connected to the rest of the brain only through a small volume of centrally running fibres beneath the auditory area. *a*, simultaneous records from two pairs of leads, as shown in diagram, of responses to laboratory noises; *b*, records from the same leads of responses to the tick of a metronome. (When isolation was completed the area became silent.)

was that the surface examined contained the auditory area (Bremer & Dow, 1939) and the laboratory in which these experiments were carried out was noisy. Fig. 6 shows records obtained from the auditory area when this area is isolated from the remainder of the cortex, but is still connected with other parts of the central nervous system. Many of the 'spontaneous' potentials recorded (Fig. 6*a*) were clearly produced by sounds in the laboratory. Fig. 6*b* demon-

strates the responses of the same preparation to the sound of a metronome. All this 'spontaneous' activity disappeared directly the area which had already been isolated around its borders was completely isolated by undercutting the remaining central connexions.

We may conclude then, that nerve cells in the parietal cortex of the chloralosed cat show a synchronized activity which is partly transmitted to them from other parts of the cortex and is in part a consequence of their direct connexions with deeper parts of the brain. Neurological isolation of this area brings to a sudden end all signs of such activity, although the degree of manipulation necessary for cutting the nervous connexions of the area does not appear otherwise to disturb the function of its cells.

Responses to single electrical stimuli

The response of isolated cortex to stimulation through an electrode placed upon its surface varies with the stimulus strength, the area excited by the electrode and with the frequency of stimulation. In the experiments described below, the stimuli were repeated at 0·5 or 1 min. intervals; at such low frequencies of stimulation the response appeared to be independent of frequency and neither fatigue nor facilitation was apparent.

The surface negative response

Using stimulating electrodes applied to the surface of the cortex and recording electrodes placed as near to these as was possible (1 or 2 mm. away), it was found that the first detectable response to a threshold stimulus was a local surface negativity. This local response is short-lived and has decayed to half of its peak value in about 30 msec. (Fig. 9*a*); it was nearly monophasic when recorded from two surface points, and was therefore not transmitted unchanged across the surface of the cortex (see also Adrian, 1936). As the strength of the stimulus was increased the peak value of the surface negative response increased, so that the relation between the two was almost linear over a wide range of stimulus strengths. Ultimately, however, increase of stimulus, beyond some five times threshold strength, produced no further increase in the response (Fig. 7). The magnitude of local responses greater than 60–70% maximal became more difficult to define because of the appearance of a second type of response in which the cortical surface became positive to unexcited tissue; this new response sometimes distorts the surface negativity, but frequently the surface positive response occurs with sufficient latency to allow precise measurement of the preceding negative response to strong stimuli (Fig. 9*b*). There seemed no doubt, where precise measurement was possible, that the surface negative response rose to a maximum with increasing stimulus strength, beyond which point the response was independent of the strength of stimulus used.

The records show that the main surface negative response is concentrated beneath the stimulating electrodes and subsides where it arose without being transmitted as such across the cortical surface. A pure surface negative response beneath the electrodes is, however, accompanied by potential changes at points up to 10 mm. away; I found that the distribution of surface potential around the central negative response was extremely complex and varied in an unpredictable fashion from one experiment to another, if the isolated slab of cortex contained sulci. It seems that the complex distribution of negative and positive potential around a stimulated point is due to a simple surface negative response which spreads to involve structures in the sulci which lie deep to the recording electrodes; for, if the isolated slab of tissue is made smaller than that

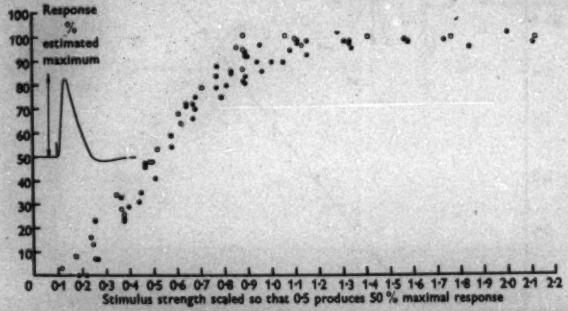

Fig. 7. The relation between peak surface negative response at about 2 mm. from a stimulated point, and stimulus strength. The points are the results of nine experiments with four cats. (□ represents measurements from monopolar records, the remaining measurements were from bipolar recording.)

illustrated in Fig. 1, so that the area contains no sulci, these complicated patterns disappear. It was found possible to isolate a strip of cortex about 6 mm. broad and 20 mm. long, running along the crest of the median suprasylvian gyrus; the cut with the bent wire was made along the edge of the sulci, limiting this gyrus so as just to exclude these sulci from the isolated strip.

In preparations of this sort it was found that the negative potential response declined progressively with increasing distance from the stimulated point (Fig. 8). The peak negative response to a stimulus appears a little later, the farther is the recording lead from the point stimulated; the wave of surface negativity seems to spread outward with an approximate velocity of 2 m./sec. Fig. 8 shows the variation of the maximum negative response with distance from the point of stimulation.

The simplest interpretation of this distribution of surface potential is that the stimulus excites beneath the electrodes a random arrangement of cell

processes which lie close to the cortical surface. A maximal negative response at the electrodes would then be obtained when the stimulus was of sufficient strength to excite all processes lying beneath them. The rapid attenuation of the surface negative response with increasing distance from its origin suggests

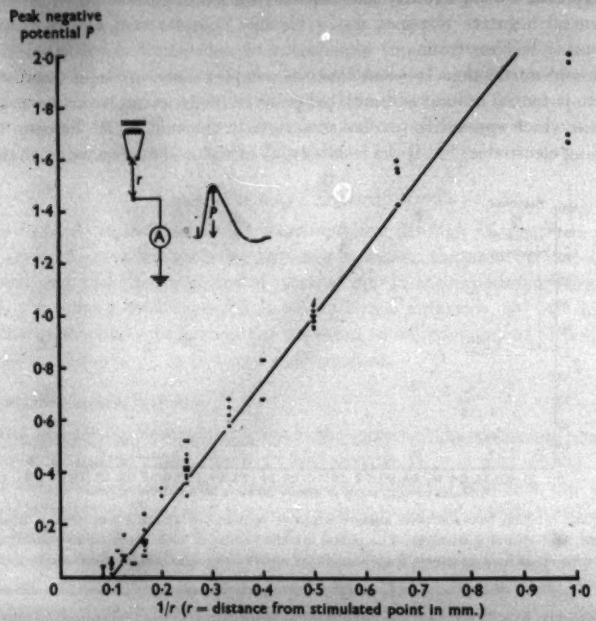

Fig. 8. The relation between peak surface negative response P to a variety of submaximal stimulus strengths, and distance r from the stimulated point on the surface of a gyrus. The points are the results of ten experiments on three cats. P is plotted in units chosen so that the mean value determined in any one experiment is unity when $r=2$ mm. The straight line has been fitted by eye and is defined by the equation

$$\frac{P}{P_{r=2}}=2{\cdot}5\left(\frac{1}{r}-\frac{1}{10}\right).$$

that synapses are not involved, and that the response spreads radially outward along those conductors which happen to pass beneath the stimulating electrodes, while the fact that no potential change can be detected at distances greater than 10 mm. from the stimulated point suggests that the longest of these conductors stretches 10 mm. across the cortical surface.

Only histological study can produce direct confirmation of these assumptions. However, it is of some interest that, assuming *all* of the conductors responsible

for the negative response stretch 10 mm., it is possible with few additional assumptions to explain the complete distribution of surface potential. If the

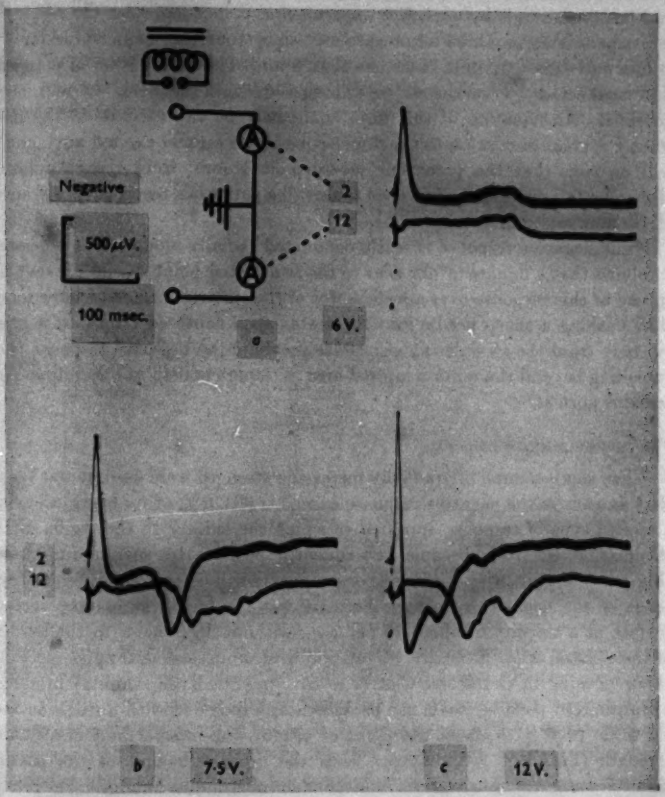

Fig. 9. Responses to surface stimulation of the isolated cortex at 2 and 12 mm. from a stimulated point (monopolar recording). *a*, local negative response to a stimulus of 6 V. applied to wicks; *b* and *c*, surface negative and spreading surface positive responses at the same points when the stimulus strength was increased to 7·5 and 12 V. respectively.

assumptions suggested in the appendix to this paper are accepted, we should expect the experimental data for peak negative potential P, when plotted against the reciprocal of distance $1/r$ from the stimulated point to fall upon

a straight line described by $P = K(1/r - 1/l)$, where l is the distance from end to end of all conductors responsible for the response, and K is a constant. The line drawn through the experimental points in Fig. 8 supposes that $l = 10$ mm. and has been fitted 'by eye'. The experimental points represent the results of ten experiments on three adult cats and show that the mean value for l is unlikely to exceed 12 mm. or be less than 8 mm. The distribution of potential was unaffected by variations in stimulus strength between 20 and 60% maximal. An equation of this form could only be expected to fit the data when r is great in relation to the dimensions of the central excited area, and it will be seen that the potentials recorded at 1 mm. from the stimulating electrodes fall short of the line used to describe potentials recorded at distances of 1·5 mm. and more.

If the negative response is really conducted radially along nerve processes, it follows that a minute injury near to the stimulated point should prevent the spread of this response over a wide sector of the cortical surface. I have found that pushing a sharp needle into the cortex to a depth of 1 mm. at a point 0·5 mm. from the stimulating electrodes prevents the negative response from spreading beyond the minute injured area, although spread in other directions remains normal.

The surface positive response

When single stimuli of gradually increasing strength were used, it was found that as soon as the negative response exceeded 60–70% of its maximal value, a second type of response appeared in which the surface in the region of the stimulating electrode became subsequently positive to surrounding tissue (Fig. 9). The properties of this response appeared to be entirely different from those of the negative response. First, it was conducted across the cortical surface at a velocity of about 15 cm./sec., without decrement, to the borders of the isolated area. Secondly, its relation to stimulus strength appeared to be all or nothing in character; that is to say, provided the stimulus produced a transmitted positive wave, the peak deflexion recorded with a single surface electrode (say 1 cm. from the point of origin) was independent of stimulus strength (Fig. 10). With bipolar leads the diphasic record of the passing positive wave was not invariably independent of stimulus strength. The reason for this is that the positive responses to weak stimuli, just above threshold, are sometimes narrow waves with a steeper rising front, whereas the responses to strong stimuli are broader waves with a slower rising wave front. Consequently, the bipolar record (which approximates to the first differential of the monopolar record) may show the greatest peak-peak measurement for responses to the lower stimulus strengths and can be misleading when used as a measure of the positive response.

The surface positive waves could be initiated by summation of two sub-

threshold stimuli. If two stimuli were applied to the cortex each only strong enough to cause, say, a half-maximal surface negative response with no subsequent positive response, a transmitted surface positive response was produced when the interval between them exceeded 5 msec. and was less than 11 msec. (Fig. 11). The precise limits to the intervals necessary for successful summation of submaximal stimuli depend, of course, upon the strengths of the stimuli used. Summation with just submaximal stimuli has been obtained at intervals as great as 150 msec.

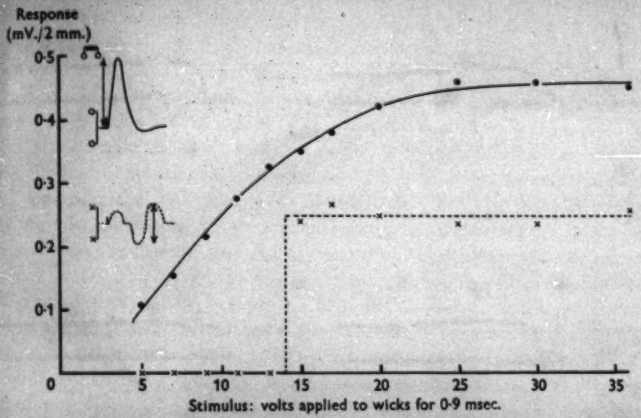

Fig. 10. The relation between the surface negative response, the surface positive response and stimulus strength. ●——●, surface negative response recorded at 3, 5 mm. from stimulus; ×------×, surface positive response recorded at 9, 11 mm. from stimulus.

Fig. 11 also shows that two stimuli which do not produce a propagated positive response, can produce a local positive response. In the same way, single stimuli which are just below threshold for the propagated response will produce a weak static positive response. The magnitude of the positive response only bears a strictly all-or-nothing relation to stimulus strength when it is recorded at some distance from the point of origin.

DISCUSSION

The isolated cortex, when set up in the way I have described, shows no sign of the mass synchronized activity of neurones seen in normal chloralosed cortex. Despite this electrical silence, the preparation can be shown to be alive; it responds to both electrical and 'physiological' stimuli. Indeed, with the crude tests of normality at present available, I have been unable to demonstrate any change in function produced by the procedure of isolation.

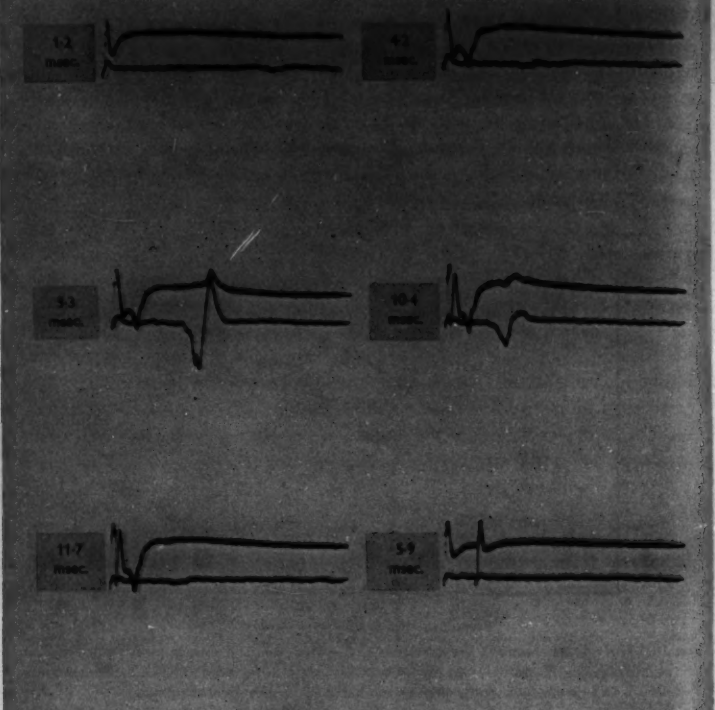

Fig. 11. Records showing the surface positive response produced by the temporal summation of two submaximal stimuli. Each pair of traces was recorded simultaneously from leads at 2, 4 and 15, 17 mm. from the stimulus. Either stimulus alone produced a surface negative response only. When the interval between the two stimuli lay between 5·3 and 10·4 msec. a propagated surface positive response was produced.

The complete absence of recordable spontaneous activity provides ideal conditions for the detailed study of responses to electrical stimuli. Admittedly, the preliminary experiments described have not done much more than confirm, under considerably easier working conditions, the basic conclusions of Adrian (1936) working with the intact brain. It appears that the simplest response of the cat's cortex when a single electrical stimulus is applied to the surface, consists in a local surface negativity beneath the electrodes. The major part of this negative response is restricted to an area within 2 mm. of the excited point, and ultimately dies where it arose after some 50 msec. The distribution of potential across the cortical surface can be explained by assuming that the stimulus has excited some of a system of nervous processes, which pass in all directions immediately beneath the stimulated point; the excitation wave spreads radially outwards with a velocity of some 2 m./sec. along these conductors until their end is reached; no synapses appear to be involved. The information which has been collected so far, is consistent with the view that these conductors all stretch approximately 1 cm. from end to end in a superficial cortical plane and seem to be distributed within this plane entirely at random, so that the response spreads equally in all directions. Increase of stimulus strength ultimately produces a maximum negative response and further increase of strength (up to three times maximal) produces no further increase of response, presumably because all the cells accessible to the stimulating current are being excited. But provided the negative response exceeds 60–70% of its maximal value, a response of entirely different properties appears. The stimulated point goes suddenly positive to surrounding tissue, and a wave of surface positivity travels outward from the centre at 10–20 cm./sec. If the positive wave appears at all at a point 4 mm. from the stimulating electrode, it has a constant size for all stimulus strengths and travels without decrement in all directions to the borders of the isolated area. The positive response must, unlike the negative response, travel either in an 'infinite' network of intercommunicating fibrils, or more probably, traverse synapses in its progress, since it does not decrease as it travels.

These surface positive responses to single stimuli are almost certainly identical with those described by Adrian (1936) as occurring with repeated surface stimulation. Adrian showed that these responses were occurring in cells lying deep to the surface, and he believed their response to be due to direct current spread from the stimulating electrodes. Although this can no doubt occur, the last experiments described, with the summation of two subthreshold stimuli, suggest the presence of at least one synapse lying in the path of excitation between the stimulating electrodes and the deep tissue responsible for the surface positive response.

The fact that all surface negative responses exceeding 60–70% of the maximal value are accompanied by a surface positive wave tempts one to assume that

with single stimuli, the superficial neurones responsible for the surface negative response are the direct source of excitation of the deeper lying neurones which give rise to and transmit the positive wave.

Experiments with partially isolated cortical slabs have shown that the responses to electrical stimulation are in some ways similar to those which result from physiological excitation transmitted to the isolated piece of brain by way of a connecting bridge. Two types of wave can be recognized when the spontaneous rhythm of undamaged brain is allowed to excite the 'isolated' cells through a gateway of normal cortex. The first is complex in form and attenuates rapidly with increasing distance from the gateway. The second type consists of large waves of surface positivity which travel across the 'isolated' area without attenuation at a velocity of about 15 cm./sec. Although it is certain that electrical stimulation of the surface provides a highly artificial method of exciting cortical neurones, it is also interesting to compare the responses obtained in this way with those obtained by Bartley & Bishop (1933). They recorded responses from the striate area of anaesthetized rabbits, while stimulating the optic nerve and observed that 'when a single stimulus calls forth a just maximal response, not all the fibres of the optic nerve have been stimulated'. The response which they were describing was found to be monophasic (i.e. static) and easily elicited by weak stimuli to the optic nerve. Greater stimulus strengths produced a diphasic response whose first phase was positive. The similarity between these observations and the responses of isolated cortex to electrical stimulation suggests that the results of electrical excitation may sometimes simulate normal activity. If it is true that all afferent impulses to the cortex first reach its most superficial layers (cf. Lorente de Nó, 1934) then the normal pathway into the cortical network would be the route most accessible to electrical stimulation.

The results of these experiments necessitate a re-examination of the origins of gross spontaneous rhythm in the anaesthetized cortex; their bearing upon the origins of normal activity in the unanaesthetized brain cannot, of course, be more than suggestive. It is often assumed that part at least of the spontaneous activity observed in the intact cortex is the result of rhythmic activity in cortical cells which are receiving no afferent impulses from an outside source (Adrian & Matthews, 1934*a*, *b*; Gerard, 1936). Two explanations of this untimulated rhythmic activity have been offered. It has been suggested that some structure in the cortex, when left at rest, can build up excitability until the moment at which it discharges spontaneously, and thereby becomes a pace-maker for surrounding neurones. Alternatively, it has been suggested that excitation finds its way into a ring of neurones around which it can run indefinitely, giving rise to cyclical stimulation of nearby tissue. The complete absence of spontaneous rhythm in the isolated cortex of the chloralosed cat does not support the former view. Dusser de Barenne & McCullock (1941) have shown that for

maintenance of spontaneous cortical activity the cortico-thalamic connexions must remain intact. My own experimental results are consistent with this conclusion; they could be held to support the hypothesis of a circular path of self-re-exciting neurones running from cortex to thalamus and back to thalamus. So far as I know, however, no unequivocal experimental evidence of such circular pathways anywhere in the central nervous system has yet been reported. In the absence of further information it seems simpler to adopt the view that spontaneous rhythm in the normal chloralosed cortex is a complex of local responses to incoming stimuli, and probably of after-discharges transmitted across the cortical surface. This assumption would be no more than a modification of the view that spontaneous activity is largely determined by a cyclical rise and fall of excitability in certain neurones which become pace-makers. It would suggest, however, that no neurone can become a pace-maker unless it has recently been excited from an outside source. This view would certainly fit the facts available; but although it gives an adequate explanation of the activities of lightly chloralosed cortical cells, we must remember that the behaviour of unanaesthetized neurones may prove quite different.

APPENDIX

The expected distribution of the surface negative response

The experimental data suggest that the longest of the conductors responsible for the surface negative response stretch so that their extremities are separated by about 10 mm. measured across the cortical surface. Suppose that through a small volume beneath the stimulating electrodes a number N of these conductors pass in all directions parallel to the cortical surface. Then stimulation of n of these conductors will cause excitation to spread along them, radially outward from its point of origin until it reaches the end of each. The density of excited conductors will clearly be proportional to the reciprocal of distance from the stimulated point.

Assuming that the action potential has the same magnitude p at any point along one of these conductors, the potential recorded at any distance r from the central point of stimulation would be kpn/r (k is a constant), provided that the conductors were distributed at random within the superficial layer and if their length were infinite. If the conductors all have a length l, then the number which will stretch a distance not less than r from the stimulated point will be $N[(l-r)/l]$. Therefore when n conductors are excited by the stimulus we should expect to record a potential, P at distance r from the stimulated point, where

$$P = \frac{kpn}{r} \times \frac{(l-r)}{l} = K\left(\frac{1}{r} - \frac{1}{l}\right),$$

where K is a constant for a fixed stimulus strength. This equation predicts that when peak negative potential is plotted against $1/r$ the data should be fitted by a straight line cutting the $1/r$ axis at $1/r = 1/l$ (Fig. 8). The equation is of course only applicable provided r is great in comparison with the dimensions of both the central excited area and the recording electrode.

SUMMARY

1. In cats anaesthetized with chloralose, part of the cerebral cortex in the parietal region has been isolated from all connexion with either the central nervous system or the adjacent cortex. The blood supply of this slab of tissue was its only connexion with the rest of the animal.

2. Neurological isolation of the chloralosed cortex caused the complete disappearance of all spontaneous electrical activity.

3. If a narrow bridge of cortical tissue connected the otherwise isolated piece of cortex to the rest of the brain, spontaneous waves entered across the bridge and excited the 'isolated' cells.

4. Electrical stimulation of the cortical surface within the isolated area excited responses similar to those obtained in the intact anaesthetized cortex.

5. A single weak electrical stimulus produced a response in which the stimulated point went negative to surrounding tissue. The magnitude of the negative response increased with increase of stimulus strength until a maximal response was attained; increase of stimulus strength beyond this point produced no further increase of response.

6. The negative response spread out from the stimulated point at about 2 m./sec. but attenuated rapidly with increasing distance from the centre and disappeared completely after travelling about 10 mm.

7. The properties of the negative response can be explained by assuming that the stimulus has excited some of a limited number of identical conducting elements, which are about 10 mm. from end to end and which lie at random in a superficial cortical layer.

8. Single negative responses which exceeded 60–70% of the maximal value were followed by a second response in which the cortical surface beneath the stimulating electrodes went positive to surrounding tissue.

9. The positive response spread out from the stimulated region at about 15 cm./sec., without attenuation to the borders of the isolated area.

10. There was an 'all-or-nothing' relationship between stimulus strength and the magnitude of the positive response.

REFERENCES

Adrian, E. D. (1936). *J. Physiol.* **88**, 127.
Adrian, E. D. & Matthews, B. H. C. (1934*a*). *Brain*, **57**, 355.
Adrian, E. D. & Matthews, B. H. C. (1934*b*). *J. Physiol.* **81**, 440.
Bartley, S. H. & Bishop, G. H. (1933). *Amer. J. Physiol.* **103**, 159.
Bremer, F. & Dow, R. S. (1939). *J. Neurophysiol.* **2**, 308.
Burns, B. Delisle (1949). *J. Physiol.* **110** *P.*
Dusser de Barenne, J. G. & McCulloch, W. S. (1941). *J. Neurophysiol.* **4**, 304.
Gerard, R. W. (1936). *Cold Spr. Harb. Symp. quant. Biol.* **4**, 292.
Lorente de Nó (1934). *Jb. Psychiat. Neurol.* **45**, 381.

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INTERACTION BETWEEN LIGHTS OF DIFFERENT WAVE-LENGTH IN THE CENTRAL FOVEA

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The general phenomenon of simultaneous contrast is of course well known. There is also a considerable literature on the effects of various adjacent secondary sources of light on the perception of colour and brightness of the primary field (e.g. Luckiesh, Taylor & Holladay, 1925; Holladay, 1926; Roaf, 1932; Stiles, 1929; Beitel, 1936; Schouten & Ornstein, 1939). Stiles & Crawford (1933, 1934), in particular, have investigated the effects of background colours and of adjacent glare sources on the least perceptible amounts of different wave-lengths on small fields, centrally fixated. Most of the measurements in the past have, however, been made with fairly large fields, or without particular reference to the part of the eye involved or when other factors were present to affect the behaviour of the so-called rod-free area in a more or less unknown manner.

The realization that the central fovea behaves as a diodic system and, functionally at least, seems to depend upon two types of receptor and two independent pathways introduces new possibilities in investigating how 'contrast' and 'adaptation' phenomena are brought about, especially in so far as these occur within the foveal centre. By using light from the spectral extremes it is possible to stimulate one of these groups of receptors very much more than the other, and by this means it can be ascertained whether both receptors produce the effects, or whether the activity of one receptor is more influenced by the activity of its own fellows or by those of the opposite type.

Beitel (1936) has shown that, with white light, the presence of an adjacent field is capable of altering the apparent brightness of a small (10′) field when both are located within the foveal area. The adjacent or secondary field in this case subtended 10′ at the eye, and its centre was situated at a point 20′ away from the centre of the primary field. At very low intensities of the secondary field the threshold for the primary field was slightly lowered, but as the intensity was increased this 'facilitation' was turned into an 'inhibition' and the

threshold was raised. Moreover, he showed that the extent to which the threshold was raised increased in proportion to the logarithm of the intensity of the secondary field. Now white light probably stimulates both foveal pathways about equally, so in these experiments there was nothing to indicate whether the effects on the two groups of receptors were mutual or not, and it seemed obviously desirable to carry out a similar investigation by using light from the spectral extremes and also to inquire more closely into the spatial characteristics of the effect.

METHODS

The apparatus used was that already described (Willmer, 1949*a*). Two circular fields, each subtending 10′ at the eye and with their centres normally separated by 20′ were viewed through an artificial pupil, 2 mm. in diameter, and were centrally fixated. One field (the test field or primary field) was reduced in brightness to threshold level and the other (the pilot or secondary field) was varied between 'threshold' brightness and several thousand times threshold brightness. Intensity control was effected either by neutral filters (Ilford) or by moving the sources of light upon the graduated scales. Colour was determined by Ilford spectral filters (601–609). The distance apart of the primary and secondary fields could be varied by the use of appropriate stops. The surrounding field was completely dark and no readings were taken till the subject had been in the dark for 15 min.

The criterion adopted throughout for 'threshold' was that the test light was just visible with direct fixation and remained visible when the pilot or secondary field was fixated. Care had to be taken, particularly with high brightnesses of the red pilot field, that the readings for direct fixation were not artificially raised by after-effects (direct adaptation) caused by the brighter fixation light. Frequent repetition of readings with suitable pauses between readings helped to minimize these effects. Nevertheless, as will be discussed later, the effects of direct and indirect adaptation are not at all easy to disentangle.

RESULTS

Before describing the results of these experiments in detail it is necessary to emphasize the peculiar variability in all the records, both in individual people at different times and between different individuals at the same time. It has not so far been possible to relate this variability with any one particular factor, but undoubtedly the degree of accuracy of fixation has, among other things, a very important bearing on the results obtained. In a later section (p. 75) various factors will be discussed in relation to the extent to which they might influence the measurements which form the basis of this paper.

The variability is well seen in the first series of experiments which was repeated after an interval of almost 2 years on the same subject. In this first series the effect of an adjacent source of red light was tested on the threshold measurements for other spectral lights. The adjacent (secondary) red light was first reduced in intensity to the minimum necessary for it to act as a fixation point, and the threshold for the different wave-bands illuminating the primary field were determined. The red light was then increased in intensity thirty-two times and the thresholds redetermined. From Fig. 1 it is clear that the presence of an adjacent red light raised the threshold for other colours progressively more and more as their wave-lengths became shorter. The violet threshold

was raised most. When the experiment was first performed the magnitude of the effect appeared to be much greater (curve *C*) than it subsequently turned out to be after frequent repetition. The relative effect at different wave-lengths, however, remained similar.

In Fig. 2 are seen the effects of different intensities of the adjacent red light on the extent to which the thresholds for violet and red are raised for different observers. For most observers the threshold for red is clearly less altered than that for violet, but the extent of the change is very variable among different

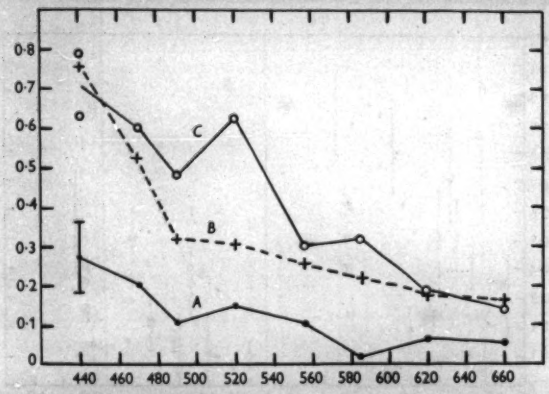

Fig. 1. Diagram showing the influence of an adjacent red field on the threshold for test fields of other wave-lengths. Ordinates: logarithm of multiples of threshold energy of the test field determined when the red field is reduced to about three times its absolute threshold and is then acting as a fixation point. Abscissae: wave-length of the test field (mμ.). *A*, ●———●, effect of a red light, ×32, as strong as the 'threshold' red (32*T*); *B*, +·····+, effect of a red light, ×288, as strong as the 'threshold' red (288*T*); *C*, ○———○, the first estimate obtained for the effects of 32*T* by the same subject who eventually gave the series of ten readings for each point the mean of which is shown in curve *A* and for which the standard deviation for the violet point is shown by the vertical line.

observers. In one case shown in the diagram, and in one other on whom some preliminary readings were taken, there was found to be no difference between the effect of red on red and that of red on violet. In another subject, who incidentally was a protanope, the difference was much greater than any of those shown in the diagram. It is thus obviously very difficult to assess the meaning to be attached to such results but there seems to be fairly clear evidence that for the majority of people the presence of an adjacent secondary source of red light raises the threshold for violet to a greater extent than it does for red. The order of magnitude of the change of the violet threshold is about the same, and of the red threshold rather less, than the change in the threshold for white

under the influence of a secondary white field as recorded by Beitel (1936). His 'inhibitory' effects were relatively greater at high intensities of the secondary source, with some facilitation at the lowest level, while in the experiments here reported the effects on the violet in particular seem to be relatively greater at low intensities than they are at the higher levels. It may be concluded, therefore, that the juxtaposition of two fields in the fovea may result in the suppression of the weaker by the stronger, and this suppression is exerted most strongly when the weaker field is illuminated by light of short wave-length and the brighter field by light of long wave-length.

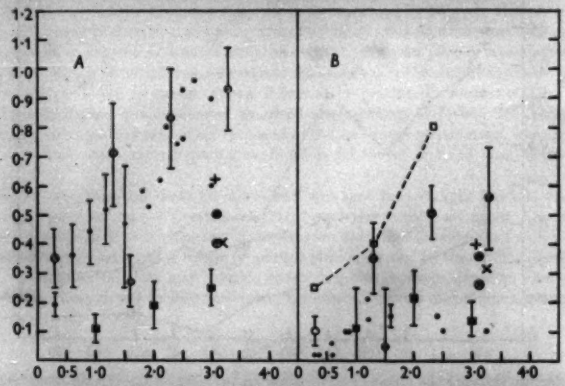

Fig. 2. Diagram showing the effects of an adjacent red source at different intensities on the threshold for (*A*) violet and (*B*) red. Ordinates: logarithm of the multiples of the energy of the test field required when the red field was at its minimum brightness. Abscissae: logarithm of the intensity of the red field. Vertical lines indicate standard deviation of the points. The different signs indicate different observers, and are the same in *A* and *B*; broken line indicates comparable figures found by Beitel (1936) for the influence of a white adjacent field on a white test field.

In one observer, in whom the differential effect between red and violet was fairly well marked, experiments were carried out in order to see how the extent of the separation between the two fields affected the result; a large series of readings of violet thresholds were taken. The readings for each position of the fixation point were taken on several different occasions and the adjacent red field had a standard brightness of about 1000 times threshold. The results are plotted in Fig. 3. It is clearly impossible to say exactly what is the relationship between the distance between the two fields and the raising of the threshold, but it is evident that the nearer the two fields approach each other the more is the violet 'occluded'. What happens when the two fields actually overlap could not be investigated with the apparatus and technique employed, but

presumably it must be related to the amount of violet light which it is necessary to add to red before the effect becomes noticeable as a change of brightness. Problems of heterochromatic matching are, however, at once involved, and when such matches are made with lights from the ends of the spectrum, difficulties immediately arise because, in the centre of the fovea, changes of brightness involve changes in hue. For example, a bright red may be made to match an orange and a bright violet becomes indistinguishable from a blue-green.

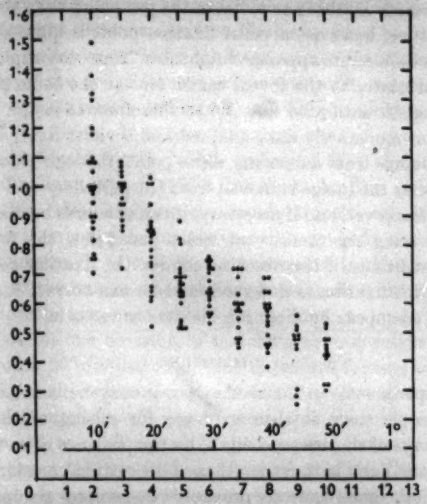

Fig. 3. The effect of an adjacent red field (× 1000 'threshold') on the threshold for violet, when the red field is placed at different distances from the violet test field. Ordinates: logarithm of multiples of the intensity of the violet field required to reach threshold when the red field was reduced to its minimum. Abscissae: distance of the centre of the test field from the centre of the red field in mm. at 70 cm. from the eye, and angle subtended at the eye.

Presumably therefore it should be sufficient to find the relative amounts of red and violet light which it is necessary to add to small fields of red or violet in order to produce any perceptible difference either of hue or brightness. One or two preliminary experiments along these lines indicated that perhaps the amount of violet necessary was the greater, but the results were not significant.

The observations so far described have all been made on the effect of red on the thresholds for other colours. Violet, when used in place of the red as a fixation light, was not found to have a comparable differential effect on the

thresholds for other colours, though there were indications of a slightly greater effect on the thresholds for the longer wave-lengths. A bright violet was not available for studying the effects at high intensity but certainly, at intensities at which a red light would exert a definite 'occluding' effect on an adjacent violet, a violet light caused almost no change in the red threshold. Actually the lowest readings for the thresholds for all colours were obtained when a violet fixation point was used. At first sight the use of a violet fixation point for investigation of the foveal centre seems not to be a good idea, since the eye is so much more sensitive to violet in the periphery than it is in the foveal centre. In practice, however, a violet fixation point is quite satisfactory, for central fixation because its apparent brightness drops very rapidly as its image falls nearer and nearer to the foveal centre and at the same time it acquires a very characteristic saturated hue. When therefore, as in the present experiments, the eye is moderately dark-adapted and a violet fixation point is used, it is found to change from a dazzling white point, through blue to a very dark saturated violet as the image is moved from the periphery and approaches the foveal centre. Moreover, and if necessary, it can of course be made to disappear completely by using an intensity of violet just below the foveal threshold. Under these conditions, if the observer can see the fixation point at all, then he knows that his fixation is not good, and he can correct it by causing the violet point to disappear by directing his gaze towards it.

DISCUSSION

From these experiments, so far as they go, it may be concluded that in the majority of people there is clear evidence for raising of the threshold, in particular for rays of short wave-length, by the presence of an adjacent source of red light. Violet light, in the strength used does not have so large a differential 'occlusive' action. Since there are probably two receptor groups in the central fovea, there is a strong suggestion that the 'red' receptor tends to suppress the activity of adjacent 'blue' receptors more extensively than it suppresses neighbouring 'red' receptors. The effect is probably retinal, rather than central because the simultaneous shining of a bright red light into one eye did not have any significant effect on the threshold as measured by the other eye. The usual (2 mm.) artificial pupil was used throughout and presumably eliminated any effects from the pupil reflexes. Crawford (1940) has similarly observed that shining a bright light into one eye does not affect thresholds determined by the other. The 'occlusive' action of the red on the violet definitely diminishes with increasing distance between the two fields, but when the red light has an intensity about 1000 times threshold the effect can be detected within an area having a radius subtending about 1° at the eye. Histologically, therefore, the machinery is presumably to be sought in the horizontal cells, amacrine cells, or ganglion cells rather than in the receptor cells or bipolar cells.

Before, however, theory is pursued too far, it will be desirable to call attention to a variety of observations, many of which have been made during the past few years in the course of investigations into the mode of action of the foveal centre, in order to indicate the sources of error which have to be assessed in evaluating effects recorded for 'small-field' measurements.

In the first place, the chromatic aberration of the eye was uncorrected in these experiments and the effects on small fields are very striking; for example, minute head or eye movements are sufficient to cause the displacement of the images of red and violet points, as used in the experiments described above, so that their apparent distance apart on the retina may be greatly changed. This is probably one major cause for the large scatter of the readings shown in Fig. 3. Secondly, if the eye is focused on the one point, say the red, then the violet point will appear relatively blurred, and will presumably be stimulating a larger area of the retina. It is true that there exists a general relationship applicable to the foveal centre, which indicates that the area stimulated multiplied by the light intensity is a constant. At first sight it might be assumed that this relationship would counteract the effects of chromatic aberration, but it is only approximately true so long as the areas do not exceed 7′ subtense in diameter. Thus with the field sizes used in these experiments the variation in size of the images when the eye is focused on either the red or the violet are sufficient to introduce errors of some magnitude. As an example of this, it was noted on one occasion in matching even a yellow-green against a red standard on a 20′ divided field, that if the eye focused on the red half, then the intensity of the red necessary to match the yellow-green was 6·8 units; while if the eye focused on the yellow-green side then the reading for the intensity of the red was 9·0 units. If the observer then tried to make the dividing line between the two fields as sharp as possible, the reading for the red was 7·6 units. It is obvious, therefore, that one particular criterion must be adopted with regard to fixation and focus and then maintained as rigidly as possible.

Another curious observation along somewhat similar lines, and one which seemed to produce a quite meaningless result, will again serve to emphasize some of the difficulties inherent in measurements dependent upon small fields. It was decided to compare the amount of light passing through a red filter from a standard source with that passing through a violet filter from the same source. Since comparison with a single standard light would involve at least one heterochromatic match, it was decided to compare the transmissions of the two filters first against a red field and then against a violet field. As shown in Table 1 the results obtained with a 20′ divided field were different for the two comparison lights and also differed from those obtained when a 2° field was used. Only one of these measurements, namely the small field measurement against a red standard, agreed with the results obtained by comparing the

intensities when both were reduced to threshold level for a 10′ field. These discrepancies must surely arise through technical defects or difficulties of one sort or another though their precise origin has not been traced. A very likely

TABLE 1

Method of measuring	Ratio of brightness of violet test light to that of the red test light (V/R)
20′ field measured against violet	1·28±0·4 (13)
20′ field measured against red	0·74±0·18 (14)
20′ field by comparison of thresholds	0·64±0·13 (5)
2° field measured against violet	1·71 and 1·57
2° field measured against red	1·40 and 1·45

explanation for at least some of the inconsistencies lies in the progressive increase in sensitivity to violet as more and more of the fovea and its immediate surroundings are involved. Thomson & Wright (1947) have shown that even in the foveal centre the amount of blue required in any given colour match progressively diminishes as the match is made at increasing distances from the actual centre. Thus, in the above experiments, the larger the violet field the greater will be its apparent brightness because more and more violet sensitive areas become involved.

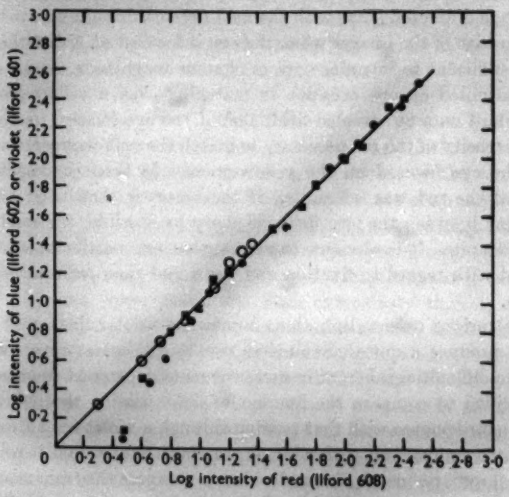

Fig. 4. The validity of heterochromatic matching between violet (or blue) and red for fields of different sizes. ○–○–○, violet on 20′ divided field; ■–■–■, violet on 2° field; ●–●–●, blue on 2° field. Note the divergence from the straight line of the points for the larger field.

A somewhat similar effect is recorded in Fig. 4. Heterochromatic matches between red and violet and between red and blue were made at one intensity

level, and then the intensity of one field was altered by a known amount and the match readjusted, by altering the intensity of the other. These two changes of intensity were then plotted against each other. It is clear from the figure that if the intensity of the one side is changed then the intensity of the other has to be changed by an exactly comparable amount. On the figure there are, however, a few points which diverge from this straight line at low intensities and for large (2°) field size. This divergence is probably due to an increased sensitivity to blue caused by the intervention of dark-adapting rods. With the small field the relationship was not disturbed by this effect.

How far the change in sensitivity to violet as the distance from the red fixation point is increased can be explained by the general change in sensitivity to violet in different regions of the fovea is uncertain. In the experiments described, care was taken to ensure that the figures for 'threshold' were those obtained as the result of both: (1) direct fixation of the violet (test) field, and (2) fixation of the red (pilot) field. That is to say, the violet was not considered to be above threshold unless it was visible under both these conditions. This presumably would mean that the final 'threshold' would be the threshold for central fixation with the red pilot field removed to different distances off centre.

Another factor which may be capable of introducing errors in small field work is the character of the fixation point. It is difficult to decide whether central fixation is better maintained by means of a very dim red light which may be slightly more easily seen by the fovea than by the rest of the retina, but which is so dim as to be difficult to pick up in an otherwise dark field, or by a bright red light which compels attention but which can obviously be seen by all parts of the eye. On the whole I have found the latter to be more satisfactory in practice, and for many observers the foveal sensitivity for red is not much different from the sensitivity in the periphery. Furthermore, the relative merits of violet and red as fixation lights have not been adequately assessed. Such considerations are clearly of the utmost importance in all work with small fields and particularly when violet and blue lights are involved, since any instability or eccentricity of fixation is bound to lead to anomalous results.

Finally, the very important question of local adaptation, both direct and indirect, in the eye as the result of fixation on red or violet points must be considered. In a previous paper (Willmer, 1949*b*), data were presented for the effects of direct adaptation to different intensities of red or violet light on the threshold for red or violet. From the evidence presented in that paper it is quite clear that, in the present series of experiments and particularly when the red pilot light was strong, the threshold measurements might be materially altered as the result of local and transitory adaptation. Moreover, the effects of adaptation to one colour on the visibility of another are by no means

simple (see, for example, Wright, 1946; Schouten & Ornstein, 1939; and Stiles & Crawford, 1933, 1934). The following brief account of some experiments on the effect of direct adaptation to red or violet on the validity of some heterochromatic intensity matches with small fields may serve to illustrate the

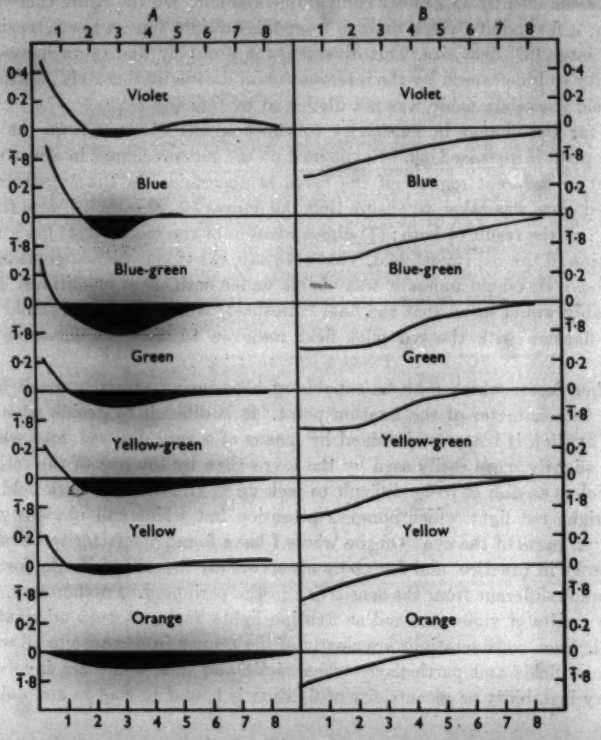

Fig. 5. Effects of direct adaptation to (*A*) red and (*B*) violet on the brightness of different spectral colours as compared with a red standard 20′ field. Ordinates: logarithm of multiples of original intensity. Abscissae: time in minutes. Shaded areas indicate that the test field is darker than the red field in spite of adaptation to red.

somewhat unpredictable results which may occur. Heterochromatic matches were set up between various spectral colours (obtained by Ilford spectral filters, 601–608) and red (Ilford spectral red, 608). The matches were made on a 20′ field divided vertically, and the field was fixated centrally and the dividing line focused. When, by altering the intensity of the red comparison field, any

particular colour had been judged to possess the same brightness as the red field the eye was then adapted to a larger (about 10°) field of uniform red (filter 608) at about 100 times threshold intensity for 3 min. The matching fields were then re-examined and the intensity of the red field altered till it again matched the coloured field in brightness. Frequent readings were taken until a steady state was again reached, which might not occur till after about 8 min. had elapsed. The results for a series of experiments with different colours are shown in Fig. 5, together with the results obtained in an exactly similar way except that violet replaced red as the adapting light. The intensity of the red light necessary to match the other colours has been plotted against the time after removal of the adapting light. As might be expected, when the field is first examined after adaptation to red all the colours (except perhaps orange) appear brighter than the red which they previously matched, but as time goes on some of the colours become significantly darker than the original red. Blue-green, for example, is particularly affected in this way, and to an extent which is almost certainly outside the range of experimental error. It is as though red light stimulates two mechanisms which are both concerned in brightness measurement. One of these recovers quickly and is important in the perception of red; the other recovers less quickly and preponderates at the blue end of the spectrum. Adaptation to violet light invariably lowered the brightness of the 'coloured' field with respect to the red field, and the two fields slowly returned to normal without any definite irregularity. The exact interpretation to be placed upon these results and indeed upon the more detailed effects of adaptation to red and to violet on the threshold to these two colours, as previously described (Willmer, 1949*b*), must await further investigation. Although the adaptation investigated was direct, effects due to indirect adaptation between the two matching fields cannot be excluded. It is certain, however, that in such measurements as those described in the experimental part of this paper great care has to be taken to eliminate as far as possible any effects caused by direct adaptation to the pilot light. In passing, it may be mentioned that the effects of adaptation on the apparent colours of the matching fields were sometimes quite dramatic; for example, when an orange field was matched in brightness with a red field and the eye was then adapted to red the orange field first appeared as a green field which subsequently became colourless, and then slowly turned yellow and finally orange.

Schouten & Ornstein (1939) showed that there appeared to be two phases in the adaptation process, a quick α-adaptation and a much slower β-adaptation. They also emphasized the fact that adaptation effects spread laterally in the retina (indirect adaptation), and that they probably involve processes occurring among the neural elements as well as those resulting from purely photochemical processes. In the experiments on the effects of adjacent red fields described above, the inhibitory, 'occlusive' of indirectly adaptive effects are

presumably nervous in origin, but direct adaptive effects may cause significant errors. A repetition of the experiments on the effects of adjacent red fields in which the fields were only exposed for brief flashes instead of continuously as in the present series might lead to different results and might help to accentuate the effects of indirect adaptation and minimize those due to direct adaptive processes.

It must be concluded from this discussion that although the foveal centre may be simpler than the rest of the eye in behaving as a diodic system, yet its investigation is beset with pitfalls for the unwary. These may have their origin in the difficulties of maintaining the requisite degree of fixation; they may be caused by the optical qualities of the small images necessary to keep within the prescribed area; finally, they may be due to influences which go on in the retina itself, of which some at least clearly interfere with the activity of areas other than those actually being stimulated by the light.

SUMMARY

1. The presence of a single red field, projected on to the central fovea, depresses the sensitivity of immediately surrounding foveal areas to all spectral colours, but progressively more as their wave-length diminishes. The presence of a violet field did not show any definite differential effect.

2. The brighter the red field the greater is the effect which it produces.

3. The effect diminishes with distance of the test field from the red field.

4. There is great variation in the magnitude of the effect between different observers, and also with the same observer at different times. Some of the numerous factors which may affect the results are discussed with further illustrations from observations on the behaviour of the foveal centre.

I am indebted to the Leverhulme Trustees for a grant enabling me to conclude these investigations while on leave of absence from University duties. I also again wish to thank all those subjects who so readily submitted to somewhat tedious and exacting conditions in order to obtain the observations recorded in this paper.

REFERENCES

Beitel, R. J. (1936). *J. gen. Psychol.* **14**, 31.
Crawford, B. H. (1940). *Proc. Roy. Soc.* B, **128**, 552.
Holladay, L. L. (1926). *J. opt. Soc. Amer.* **12**, 271.
Luckiesh, M., Taylor, A. H. & Holladay, L. L. (1925). *J. opt. Soc. Amer.* **11**, 311.
Rosa, H. E. (1932). *Proc. Roy. Soc.* B, **110**, 448.
Schouten, J. F. & Ornstein, L. S. (1939). *J. opt. Soc. Amer.* **29**, 168.
Stiles, W. S. (1929). *Proc. Roy. Soc.* B, **104**, 322.
Stiles, W. S. & Crawford, B. H. (1933). *Proc. Roy. Soc.* B, **113**, 496.
Stiles, W. S. & Crawford, B. H. (1934). *Proc. Roy. Soc.* B, **116**, 98.
Thomson, L. C. & Wright, W. D. (1947). *J. Physiol.* **105**, 316.
Willmer, E. N. (1949*a*). *J. Physiol.* **110**, 377.
Willmer, E. N. (1949*b*). *J. Physiol.* **110**, 422.
Wright, W. D. (1946). *Researches on Normal and Defective Colour Vision.* London: Kimpton.

J. Physiol. (1950) 111, 81–88 547.435.292:612.826.3

THE IMMEDIATE AND DELAYED EFFECTS OF DIISOPROPYLFLUOROPHOSPHATE INJECTED INTO THE SUPRAOPTIC NUCLEI OF DOGS

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It was previously suggested that acetylcholine (ACh.) was the transmitter somewhere in the central nervous system of impulses releasing antidiuretic hormone (ADH) from the pars nervosa of the pituitary, and later (Pickford, 1947) it was shown that ACh. could, under certain conditions, stimulate the cells of the supraoptic nuclei. The present observations were part of a series made to find whether ACh. is the normal transmitter of impulses at the supraoptic nuclei.

The acute and chronic effects of injecting diisopropylfluorophosphate (DFP) amongst the supraoptic cells were noted. As this substance combines irreversibly with choline esterase (ChE) then, if ACh. is the transmitter, the injected DFP should result in a brief inhibition of the rate of urine flow followed first, by polyuria due to the presence of an excess of ACh. and, later, by recovery to normal levels of water exchange, a chain of events that would be analogous to the muscle reactions seen by Harvey, Lilienthal, Grob, Jones & Talbot (1947) on the injection of DFP into the brachial artery of man. These workers noticed that some degree of paresis persisted for 11 weeks. Grob, Harvey, Langworthy & Lilienthal (1947) observed EEG changes after DFP in man for as long as 42 days, so it seemed probable that the time for recovery of the supraoptic neurones would be long. Assuming from the work of Pickford (1939, 1947) the release of the ADH by ACh., we tried to estimate this recovery time by giving intravenous ACh. to dogs after DFP and noting whether or not an inhibition of the rate of urine flow followed.

METHODS

The observations were made on dogs. The method of preparation of the animals, the injection of DFP into the supraoptic nuclei and the histological controls were similar to those previously described (Pickford, 1947). The only exceptions to this were that not all the animals had their

kidneys denervated and that a number of intermediate sections of the hypothalamus that would otherwise have been thrown away were stained with isamine blue, or by Rogers' silver nitrate method to follow the supraoptico-hypophyseal tracts. Pure DFP in a total dose of 120 μg. was injected at two or three sites in the supraoptic nuclei. The DFP was made up shortly before use in a part solution-part emulsion of 0·9 % NaCl and well shaken. The volume of each injection was 0·002 ml.

RESULTS

Immediate effects

The immediate effect of DFP injected into both supraoptic nuclei was observed in five dogs, three of which were allowed to survive. Two of the latter group had had their kidneys denervated 7 days before the observations

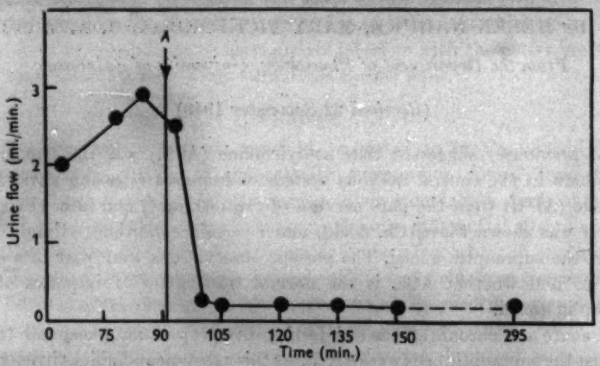

Fig. 1. The effect of the hypothalamic injection of DFP on the rate of urine flow in the chloralosed dog. Trix, weight 8 kg. At time *O*, 250 ml. water containing 1·0 mg. chloralose given by mouth. At *A*, total dose of 120 μg. DFP injected into right and left supraoptic nuclei.

were made. The animals were anaesthetized as on previous occasions (Pickford, 1947) with chloralose given by mouth in 250–300 ml. water so that in all animals but one the rate of urine flow was initially fairly high. In every instance after the injection of DFP the rate of urine flow was maximally inhibited in 5–9 min., and in four of the dogs was still inhibited after 3½ hr. In one dog, Spot, the rate rose in 1½ hr. from the low pre-injection level of 0·07 ml./min. to 1·0 ml./min. The low initial rate was perhaps due to the fact that this dog was accidentally given a larger dose of chloralose than the others. In two dogs the renal clearances of creatinine and diodone were measured. After the injection of DFP there was in one animal a slight temporary fall, and in the other a marked temporary increase in both clearances. Thus the rise or fall of the clearances was unrelated to the rate of urine flow. Fig. 1 is typical of the change in rate of urine flow seen in four of the dogs.

Delayed effects

The three dogs that were allowed to survive, two of which had denervated kidneys, and one other injected dog on which no acute observations were

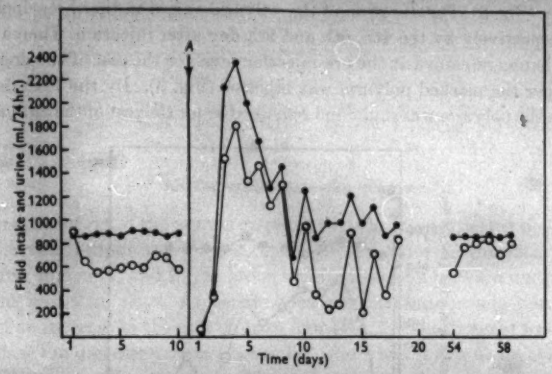

Fig. 2. Urine output and total fluid intake per 24 hr. of Spot, weight 5·5 kg. Before *A*, normal fluid exchange. On day *A*, a total of 120 μg. DFP injected into both supraoptic nuclei. ●——●, fluid intake; ○——○, urine output.

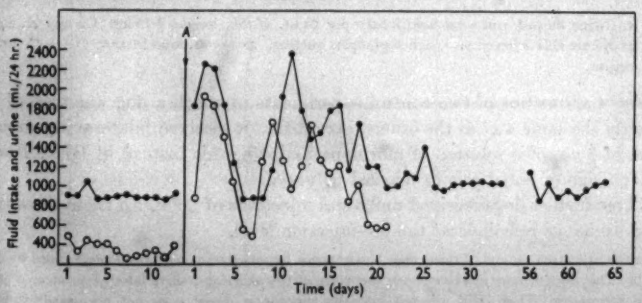

Fig. 3. Urine output and total fluid intake per 24 hr. of Snapper, weight 6·3 kg. Before *A*, normal fluid exchange. On day *A*, total of 120 μg. DFP injected into both supraoptic nuclei. ●——●, fluid intake; ○——○, urine output.

made, were placed in metabolism kennels whilst still anaesthetized and thereafter their fluid intake and urine output per 24 hr. measured for several days. At later dates the observations were repeated or the fluid intake alone measured. The animals were allowed to survive respectively for 5, 5½, 7 and 8½ months. One dog, Snapper, needs separate consideration because of the findings at

autopsy. The other three dogs, following the injection of the DFP, all had a temporary polyuria. In one this was present the day after operation, in one there was a 24 hr. period of anuria followed by a full polyuria, and in the third there was a 24 hr. period of anuria with full polyuria appearing only on the third day (Fig. 2). The duration of the polyuria varied in the three dogs, being ended respectively by the 4th, 6th and 9th day after injection. Thereafter the fluid exchange remained at the pre-injection level for the rest of the dogs' lives. In Snapper the marked polyuria was biphasic (Fig. 3). By the 19th day after injection the polyuria was slight and remained so for the rest of the animal's life.

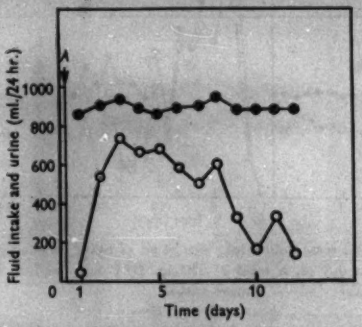

Fig. 4. Urine output and total fluid intake per 24 hr. *M*, 60, weight 7·75 kg. On day *A*, 8 μg. morphine HCl injected into each supraoptic nucleus. ●——●, fluid intake; ○——○, urine output.

Fig. 4 shows one of two control experiments in which a dog was treated in exactly the same way as the others, except that it received bilateral injections, each of 8 μg. of a solution of morphine hydrochloride instead of DFP. There was no sign of polydipsia or marked polyuria.

Three further dogs received unilateral injections of DFP. In these three the fluid exchange remained at the pre-injection level.

At the operation on one of these dogs the anterior hypothalamus was accidentally torn by the needle. This may or may not have been the cause of the abortion 2 days later of an unsuspected early pregnancy. That DFP injected into the supraoptic nuclei does not necessarily induce abortion was seen in Trix (Fig. 1) who whelped 42 days after the injection. The puppies were apparently healthy but died in 3 days of maternal neglect.

Estimation of recovery time

Table 1 shows the response after DFP to the intravenous injection of ACh. It may be seen that for a long time only rarely did ACh. cause an inhibition of the rate of urine flow, generally it led to no change at all, occasionally to an increase as will be described below. After a critical time which differed for each dog ACh. regularly inhibited the rate of urine flow in Spot, Poppet and Trix.

In Snapper the response to ACh. was feeble and inconstant till death. Two mg. of subcutaneously administered eserine salicylate appeared to reinforce the effect of ACh. in this dog on the 154th and 161st days after DFP. A similar potentiation was seen in Poppet on the 149th day, and also in normal dogs in another series of experiments. The failure of the response to ACh. has not been seen after hypothalamic injections of morphine HCl or of ACh.

TABLE 1

Dog...	Poppet		Spot		Trix		Snapper	
	Day 1–89	Day 90–210	Day 1–139	Day 140–255	Day 1–104	Day 105–150	Day 1–118	Day 119–165
No. of tests	22	19	26	8	14	6	18	7+2*
Urine flow rate inhibited	1	19	3	8	1	6	0	2+2*

* After subcutaneous eserine salicylate.

Before the return of the normal response to ACh. an unexpected phenomenon was observed in Spot and Poppet, in the former twice on the 75th and 77th days after injection, and in the latter on six occasions between and including the 18th and 23rd days. At these times the intravenous injection of ACh. induced an increase in the rate of urine flow (Fig. 5). The increase was seen not only when the diuresis was decreasing but also when it was at its height. This type of response was not seen in Trix or Snapper, and never in normal dogs.

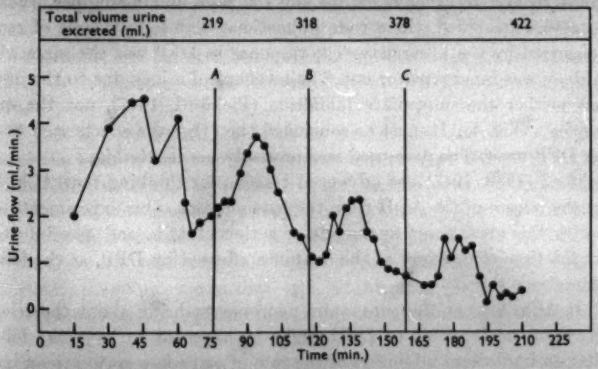

Fig. 5. Effect of intravenous ACh. on rate of urine flow of Spot, on 77th day after injection of DFP into supraoptic nuclei. At time O, 250 ml. water by mouth and 2 mg. atropine sulphate subcutaneously. At *A*, *B* and *C*, 1 mg. ACh. given intravenously.

The renal clearances of creatinine and diodone were once measured in Poppet on the 53rd day after DFP, both before and after the intravenous administration of ACh. There was a small temporary rise in both clearances with no change in the rate of urine flow.

Autopsy findings

Histological examination showed that in Spot, Poppet and Trix the injection of DFP had been well placed. The number of supraoptic and paraventricular cells was somewhat less than normal. Possibly a number of the former were destroyed by direct contact with a high concentration of DFP, and some cells of the latter nuclei underwent retrograde degeneration due to injury to their axons. These dogs had posterior lobes that were slightly shrunken but appeared normal in sections stained with isamine blue and by Rogers' silver nitrate method. In the control dogs the injection of solutions containing morphine HCl or ACh. did not result in any considerable loss of neurones. In Snapper it was found that one injection had been placed far too deeply and had passed through and destroyed the right paraventricular nucleus. The left paraventricular and the two supraoptic nuclei had a fewer than normal number of cells. The pars nervosa showed extreme atrophy.

DISCUSSION

The injection of DFP into the supraoptic nuclei of dogs caused, under chloralose anaesthesia, an immediate inhibition of the rate of urine flow, followed in 1–3 days by a marked polyuria lasting for 4–19 days. Consistently normal responses to ACh. returned in 89, 104 and 139 days, and in Snapper, whose pars nervosa was atrophied, there were indications that some degree of recovery had occurred by the 118th day. The response to DFP was the same whether the kidney was innervated or not. The mechanical injury due to the injection caused neither the immediate inhibition (Pickford, 1947), nor the marked after-effects (Fig. 4). It must be concluded then that the effects seen were due to the DFP itself. The dose used was probably needlessly high.

Pickford (1939, 1947) has advanced reasons for thinking that ACh. brings about the release of the ADH from the pars nervosa. Our experiments accord well with this idea, point to the site of action of ACh. and give information about the time of recovery of the neurones affected by DFP, as the following considerations show.

(*a*) If ACh. acts at the supraoptic neurones to bring about the release of ADH, then the injection of DFP might be expected to have the following results: an immediate inhibition of the rate of urine flow owing to an increase in the concentration of ACh. to a level that stimulates and so induces the release of ADH; polyuria due to the appearance and persistence of a paralysing concentration of ACh.; a return to a normal level of fluid exchange when 10–20 % of the normal concentration of ChE reappears in the neurones (Koelle & Gilman, 1947); no consistent inhibition of the rate of urine flow on the intravenous injection of ACh. until the neurones have recovered nearly completely. As the results show all these events occurred. The slow onset of

polyuria in Spot and Trix may have been due to a spread of DFP or of ACh. to neighbouring cells in the nuclei with a consequently persisting inhibitory stimulus, or the reason may be totally different and related to the observation of Grob *et al.* (1947) that after the administration of DFP to man it was 2–7 days before changes were seen in the EEG. The longer biphasic polyuria of Snapper was perhaps related to the injury to the hypothalamus and the atrophy of the pars nervosa.

Concerning the diuretic action of ACh. seen in Spot and Poppet we can offer only suggestions. The effect may be due to a reversal of the normal action owing to the abnormal condition of the neurones; it may be due to the unmasked activity of another set of cells; or it may be that a normal stimulus and release of pars nervosa hormones followed the injection, but so small in degree that the only effect was one on the efferent arterioles of the glomeruli, with a consequent increase in the filtration rate. It is difficult to see how the effect could be a direct one of ACh. on the kidneys in this instance alone, as a similar effect has never been seen after removal of the posterior lobe or section of the supraoptico-hypophyseal tracts. We intend to try to repeat this part of the work.

(*b*) If ACh. activates secreting cells in the pars nervosa itself, then the inhibitory response to it should be unaffected, provided always that the secreting cells are normal. The autopsy findings in three of the dogs make it reasonable to suppose that the gland was potentially normal and adequate throughout the observations, but inactive because of a functional change in the neurones supplying it. It therefore seems improbable that ACh. acts on cells in the pars nervosa.

(*c*) If the injected ACh. stimulates an unidentified set of central nervous neurones the path to the pars nervosa must run through either the supraoptic nuclei or fibres entering the pars nervosa from the posterior hypothalamus. If the former is the case then stimuli passing through the supraoptic nuclei should not be blocked unless ACh. is the transmitter here too. If the latter is the case, then there is no reason why DFP should prevent the liberation of the ADH.

Our results therefore suggest that ACh. is the normal transmitter of impulses in the supraoptic nuclei, and that it does not activate secretory cells in the pars nervosa causing them to release the ADH. This is in agreement with the findings of Feldberg & Vogt (1948) on the distribution in the central nervous system of the ACh.-forming enzyme systems.

SUMMARY

1. Experiments are described on the immediate and delayed effects of DFP injected into the supraoptic nuclei of dogs.

2. The immediate effect, under chloralose anaesthesia, was a profound and long-lasting inhibition of the rate of urine flow.

3. The delayed effect was a marked polyuria of 4–19 days' duration, with finally a return to pre-injection levels of fluid exchange.

4. Despite the establishment of normal fluid exchange the normal inhibition of the rate of urine flow on giving intravenous ACh. was not seen for 89–139 days, suggesting that this length of time was needed for complete recovery of the neurones.

5. A consideration of the results supports an earlier suggestion that ACh. is the transmitter at the supraoptic nuclei. It seems improbable that ACh. stimulates secretory elements in the pars nervosa.

We wish to express our gratitude to the Moray Fund for a grant towards the expenses of this work, and to Dr H. Adam of the Dept. of Materia Medica for allowing us to help ourselves to his supply of DFP.

REFERENCES

Feldberg, W. & Vogt, M. (1948). *J. Physiol.* **107**, 372.
Grob, D., Harvey, A. M., Langworthy, O. R. & Lilienthal, J. L. (1947). *Johns Hopk. Hosp. Bull.* **81**, 257.
Harvey, A. M., Lilienthal, J. L., Grob, D., Jones, B. F. & Talbot, S. A. (1947). *Johns Hopk. Hosp. Bull.* **81**, 267.
Koelle, G. B. & Gilman, A. (1947). *J. Pharmacol.* **87**, 421.
O'Connor, W. J. (1947). *Quart. J. exp. Physiol.* **34**, 29.
Pickford, L. M. (1939). *J. Physiol.* **95**, 226.
Pickford, L. M. (1947). *J. Physiol.* **106**, 264.

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SOME OBSERVATIONS WITH THE PHASE-CONTRAST MICROSCOPE UPON INCINERATED HUMAN BLOOD CELLS

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The technique of micro-incineration has been previously employed for studies upon the mineral constitution of blood cells (Policard, 1928*a*, *b*; Policard & Pillet, 1928*a*, *b*; Policard & Rojas, 1935*a*, *b*; Scott, 1932, 1933, 1934; Kruszyński, 1937). The distribution of the mineral ash in the incinerated cells has been observed either by means of darkground illumination or by incident light (Leitz Ultropak, etc.). The identification of metallic ions has been based mainly upon the appearance and colour of the ash in incident light.

The experiments reported in the present paper have been carried out with the aid of the phase-contrast microscope, which has been found to be very suitable for the study of incinerated preparations, and objective microchemical tests have been employed for the identification of the metallic ions of the mineral ash.

METHODS

Normal human blood provided the main material; additional investigations were performed upon blood from leukaemic and anaemic subjects and upon leucocytes in pus. Thin cover-slip films of the cells were made, dried rapidly at room temperature, and placed in the microincinerator tube. The temperature within was then gradually raised to approx. 450° C. as gauged by the colour of the glowing silica (Tschopp, 1927). Incineration was considered to be complete as soon as the dark carbonized patches in the specimen disappeared (Policard, 1929), and the specimens were allowed to cool down slowly inside the incinerator tube.

The mineral ash residues are very labile and the application of immersion oil disperses the fine particles. This, of course, precludes the use of combinations of optical components which immerse upon both surfaces of the preparation, such as high-power dark-ground condensers in conjunction with immersion objectives. In transmitted light, the residues are invisible (except where thick specimens are used). The cover-slips were accordingly mounted upon metal frames, with the ash-bearing surface downward, and examined with incident light and with the phase-contrast microscope (Cooke, Troughton & Simms, immersion objective, ×95). Monochromatic lighting was employed, the green line of the mercury spectrum being chosen.

For the study of the chemical constitution of the ash, the following tests were employed:
(*a*) For Na and K. The sodium cobaltinitrite and silver nitrate test of Feigl (1939).
(*b*) For Ca. The formation of calcium sulphate crystals after application of an alcoholic solution of H_2SO_4, a modification of the gypsum test (Kruszyński, 1934).
(*c*) For Fe. The HCl ferro- ferri cyanide test (Lison, 1936; Feigl, 1939).
Technical difficulties precluded tests for Mg, P and Cu.

RESULTS

Red blood corpuscles. The appearance of the mineral residues (spodograms) of normal corpuscles is shown in Pl. 1, fig. 1. As seen with the phase-contrast microscope, the corpuscles have retained their shape and configuration to such an extent that their appearance is virtually indistinguishable from that of a fixed and stained smear. The ash appears to be composed of extremely fine dust-like particles of equal size, which are at the limit of optical resolution. Normal erythrocytes show fluctuations in their ash content; some are well filled, including the central concavity, whilst in others this appears empty. Spodograms of hypochromic erythrocytes show a diminution in the amount of ash, the mineral salts forming but a narrow ring on the periphery of the erythrocytes.

Na and K salts are extremely hygroscopic, and spodograms kept uncovered at room temperature speedily become hydrated. Quite large droplets, presumably formed of a solution of the salts, cover the erythrocytes. Crystals may also appear. The former may become so large and numerous as to obscure the underlying structures (Pl. 2, fig. 14), or they may remain small and confined to a few cells (Pl. 1, figs. 1, 2). Upon drying such a hydrated spodogram by warming in the incinerator, crystals became apparent (Pl. 1, fig. 5). Both droplets and crystals were removed by applying a micro-droplet of distilled H_2O to the spodograms (Policard & Okkels, 1929). The resulting drop of solution gave a strongly positive reaction with sodium cobaltinitrite (Feigl, 1939) which indicates the presence of K.

Spodograms, previously hydrated either by exposure to air or by sprinkling with distilled H_2O, were rinsed in distilled water to dissolve soluble salts, dried and examined. There were obvious differences between individual cells, some of which now possessed but little ash; this finding also reflects a variation in the Na and K, and probably of iron and other mineral contents of the erythrocytes (Pl. 1, fig. 3).

Smears were exposed to the vapour of H_2SO_4 prior to incineration. According to Policard & Pillet (1928*a*, *b*) Na and K salts are thereby converted into the relatively insoluble sulphates. Spodograms of such preparations showed little propensity to become hydrated.

Cornes (1944) incinerated whole blood *en masse* and showed by chemical analysis of the ash that some of the Na and K salts volatilized at high temperature; the remaining Na and K could be removed by washing the ash in

acetic acid. Washed spodograms were therefore treated in this way; there still remained a considerable quantity of ash, composed, presumably of Fe, etc. (Pl. 1, fig. 6). In order to obtain a photograph of such a washed spodogram, it proved necessary to use wet specimens, that is, the acetic acid solution was not removed by drying; for, upon drying, the amount of ash was so slight as to be scarcely visible even with the phase-contrast microscope.

It was possible to demonstrate the presence of Ca in spodograms (previously treated to remove Na and K as described above) by the application of a drop of 2% alcoholic solution of H_2SO_4 (Kruszyński, 1934); crystals of $CaSO_4.H_2O$ are formed (Pl. 2, fig. 7). As the Ca content is known to be 0·001% of total solids (Lovatt Evans, 1947), it seems likely that the source of this Ca was the plasma around the cell; the distribution of Ca is to be described later.

Such spodograms, washed in distilled water and acetic acid, treated with alcoholic H_2SO_4 and dried, still show faint ghost-like outlines of erythrocytes (Pl. 2, fig. 7). A micro-drop of HCl and ferroferricyanide (Lison, 1936; Feigl, 1939) placed upon such cells, becomes blue immediately, revealing the presence of Fe.

No reliable evidence of quantitative differences in the Fe content of erythrocytes has been obtained so far.

Leucocytes. All types of incinerated white cells show clearly under the phase-contrast microscope, and there is no difficulty in differentiating between the various types (Pl. 2, figs. 8–13). The outlines of the nuclei, nucleoli and chromatin are visible. The protoplasm of granular leucocytes, especially of eosinophils, is visible as an accumulation of homogeneous ash more abundant midway between the nuclear membrane and the periphery of the cell, so that a halo often appears surrounding the nucleus. Whether this localization occurs in the living cell, or whether it is an artefact, is difficult to decide. Shrinkage of the nucleus and the protoplasm during incineration could account for it, but, since an ash-free zone constantly appears near the nucleus, the protoplasm in that site should evidently be regarded as at least especially labile (Pl. 2, figs. 8, 9 and 13).

The granules of acidophil leucocytes show clearly in spodograms (Pl. 2, figs. 11–13); they are not hygroscopic and they differ in appearance from the hydration droplets seen with erythrocytes.

Smears of pus are more suitable than blood films for the investigation of leucocytes, since a single field is rich in these cells. The protoplasmic ash residues of young and old polymorphs, based on the morphology of the nucleus, appeared similar. On the other hand, degenerating cells seemed either poor in protoplasmic ash content, or possessed none, and appeared as naked nuclei. They were reminiscent of, and probably identical with, the so-called 'basket' cells seen in stained preparations. Spodograms of pus frequently showed leucocytes containing mineral residues of bacteria; for instance, Pl. 2, fig. 17,

shows the appearance of two pus cells (polymorphs) after incineration. Mineral residues corresponding in appearance exactly to scattered cocci were present in the original preparation around and within these cells, but do not show convincingly in the photograph. However, the mineral skeleton of a chain of cocci within the upper cell can be seen as indicated by an arrow.

The mineral residues of monocytes and lymphocytes were less dense than they were in polymorphs, and their protoplasm was more evenly distributed.

No definite indication of the presence of azurophil granules has been encountered in the spodograms of lymphocytes.

Incinerated leucocytes are strikingly less hygroscopic than erythrocytes. The erythrocytes of Pl. 2, figs. 14, 15, are completely hydrolysed, their mineral residues forming an amorphous background, but the leucocytes are not. This suggests that their Na and K content is slight, if these substances are present at all. Furthermore, the appearance of their spodogram was not altered by rinsing in water and drying. Application of a drop of 2% alcoholic solution of H_2SO_4 to a spodogram previously washed in water led to the formation of crystals, and the complete disappearance of leucocytes. It therefore seems likely that the nuclear and protoplasmic ash of incinerated leucocytes consists mainly of Ca and perhaps of Mg salts.

Blood platelets. Incinerated blood platelets could easily be distinguished on account of their shape and size. Two kinds of their mineral salt deposits were observed in the spodograms. On the one hand, exceedingly hygroscopic deposits, and on the other, less hygroscopic (Pl. 2, fig. 16).

Plasma. A smear of blood plasma freed of cells by centrifugation, showed after incineration a considerable amount of mineral residues. In the spodograms of blood, ash deposits of the plasma frequently obliterated the outlines of the erythrocytes. A film exposed to H_2SO_4 vapour before incineration produced abundant amorphous residues representing plasma, which suggests that Na and K salts are present. The application of micro-drops of 2% H_2SO_4 to spodograms produced crystals which from their appearance were recognized to be $CaSO_4.H_2O$.

DISCUSSION

The remarkable appearance of incinerated white and red blood cells which has been obtained with the phase-contrast microscope calls for some comment, since in the past, investigations upon micro-incinerated specimens have been undertaken mainly with the aid of reflected light or dark-ground microscopy. There is no doubt that to a considerable extent the picture obtained by these latter methods is misleading; in the case of incident light in particular, larger particles reflect more light than smaller particles and the latter may be invisible as a result of a 'dazzle' effect (cf. Pl. 1, figs. 1, 4). With the phase-contrast microscope, on the other hand, a more uniform illumination is obtained, and there can be little doubt as to the superiority of the method

for problems of this nature. The results described above suggest first of all that quantitative variations occur in the mineral constitution of normal erythrocytes.

Either haemoglobin or other constituents may be involved in the variations which may perhaps be a reflexion of the age of the cell, since differences between old and young erythrocytes have been revealed with serological methods (Ashby, 1919) and by staining (Granick, 1943).

The evidence for individual variations is, first, that under the phase-contrast microscope cells not subjected to any form of treatment appear to vary in density (Pl. 1, fig. 1). In the second place, after hydration some cells possess more droplets than others (Pl. 1, figs. 2, 4). This phenomenon must be due to inherent constitutional differences between individuals, and can hardly be a function of varying degree of hydration since erythrocytes exhibiting obvious differences may be only a few microns apart on the slide.

Thirdly, after removal of Na and K by washing in water and acetic acid, differences are apparent. These facts suggest that the Na and K content of erythrocytes is variable. It is interesting in this connexion that Kerr (1937) has described variations in the Na and K content of blood of different species, orders and individuals ashed *en masse*. Hitherto, the only suggestion that variations may occur in erythrocytes has been that of Policard & Rojas (1935*a*, *b*), who consider that in the fish *Cichlasoma*, the iron content of incinerated erythrocytes varies from cell to cell. It is hoped to investigate the possibility of iron variation with the present technique.

The appearance of incinerated white blood cells has been described by Scott (1933) and Horning (1942), but neither author has described the existence of mineral residues corresponding to the cytoplasm of the lymphocytes and monocytes. Scott has even gone so far as to state that 'in the small lymphocyte the nucleus is the only portion of the cell, other than the limiting membrane, which exhibits a deposit of inorganic salt'. However, as may be seen in Pl. 2, figs. 9, 10, an obvious cytoplasmic residue has been encountered in the present investigations, which suggests also that the mineral ash of the nuclei of all white cells consists mainly of Ca salts. Scott (1933), in his very brief account of incinerated leucocytes, mentions the presence of silica, but not of calcium.

The present observations upon granulocytes suggest that, whilst there is no appreciable difference in the appearance of the ash of the cells at different stages of maturity, degenerating cells are devoid of protoplasmic ash and appear as naked nuclei.

Horning's (1942) statement that 'blood plasma leaves no detectable ash' has proved to be premature, since this ash is plainly visible and seems to be composed principally of K, Na, and Ca.

SUMMARY

1. Thin smears of normal human blood cells have been microincinerated. The mineral ash has been examined with the phase-contrast microscope, and metallic ions have been identified by microchemical tests.

2. Under the phase-contrast microscope both the erythrocytes and the leucocytes appear very similar to non-incinerated specimens.

3. Na, K and Fe were identified in the ash of erythrocytes. It appears that wide differences in Na and K content exist between normal red cells.

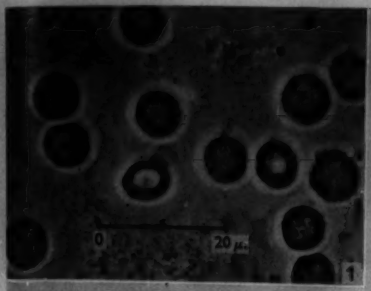
4. Lymphocytes and monocytes possess an abundant cytoplasmic ash, contrary to previous reports. The ash of these cells consists mainly of Ca.

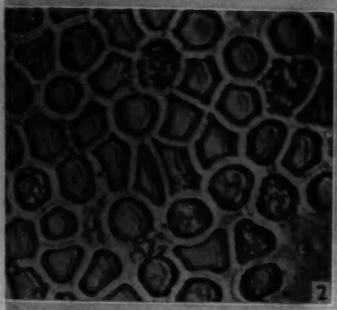
5. Both platelets and plasma leave a recognizable mineral ash after incineration.

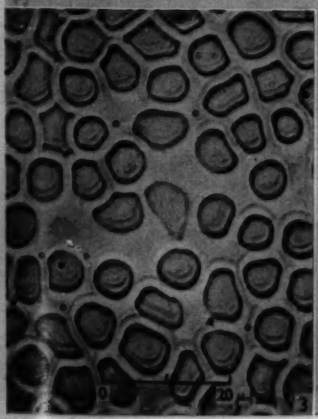
Thanks are due to Dr N. M. Hancox who furnished much valuable criticism and also suggested the use of the phase-contrast microscope; to R. McV. Weston of SIMPL, Ltd., for advice regarding photographic technique, and to C. A. McDowell for his help in constructing the microincinerator.

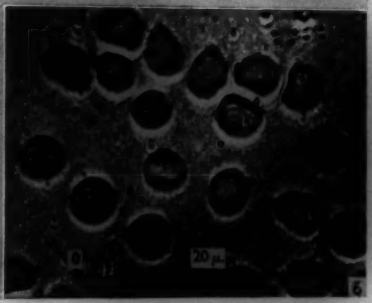
REFERENCES

Ashby, W. (1919). *J. exp. Med.* **29**, 267.
Cornes, J. J. S. (1944). *N.Z. J. Sci. Tech.* **26** B, 190. Cited in *Biol. Abstr.* 1945.
Lovatt Evans, C. (1947). *Principles of Human Physiology.* London: Churchill.
Feigl, J. F. (1939). *Qualitative Analysis by Spot Tests.* London.
Granick, S. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 255.
Horning, E. S. (1942). Bourne's *Cytology and Cell Physiology*, pp. 160-188. Oxford: Clarendon Press.
Kerr, S. E. (1937). *J. biol. Chem.* **117**, 227.
Kruszyński, J. (1934). *Bull. Acad. Pol. Sc.* B, **2**, 105.
Kruszyński, J. (1937). *Z. wiss. Mikr.* **54**, 411.
Lison, L. (1936). *Histochimie animale.* Paris: Gautier-Villars.
Policard, A. (1928*a*). *Bull. Histol. appl.* **5**, 260.
Policard, A. (1928*b*). *Bull. Histol. appl.* **5**, 350.
Policard, A. (1929). *Protoplasma*, **7**, 464.
Policard, A. & Okkels, H. (1929). *Anat. Rec.* **44**, 349.
Policard, A. & Pillet, D. (1928*a*). *C.R. Soc. Biol., Paris*, **98**, 1350.
Policard, A. & Pillet, D. (1928*b*). *C.R. Soc. Biol., Paris*, **99**, 85.
Policard, A. & Rojas, P. (1935*a*). *C.R. Soc. Biol., Paris*, **120**, 366.
Policard, A. & Rojas, P. (1935*b*). *Rev. Soc. Arg. Biol.* **164**. Cited in *Biol. Abstr.* 1936.
Scott, G. H. (1932). *Science*, **76**, 148.
Scott, G. H. (1933). *Amer. J. Anat.* **53**, 243.
Scott, G. H. (1934). *Protoplasma*, **20**, 133.
Tschopp, E. (1927). *Hdb. mikr. Anatomie* (*Möllendorff's*). Band I, Teil 1, p. 567.









Figs. 1–6.

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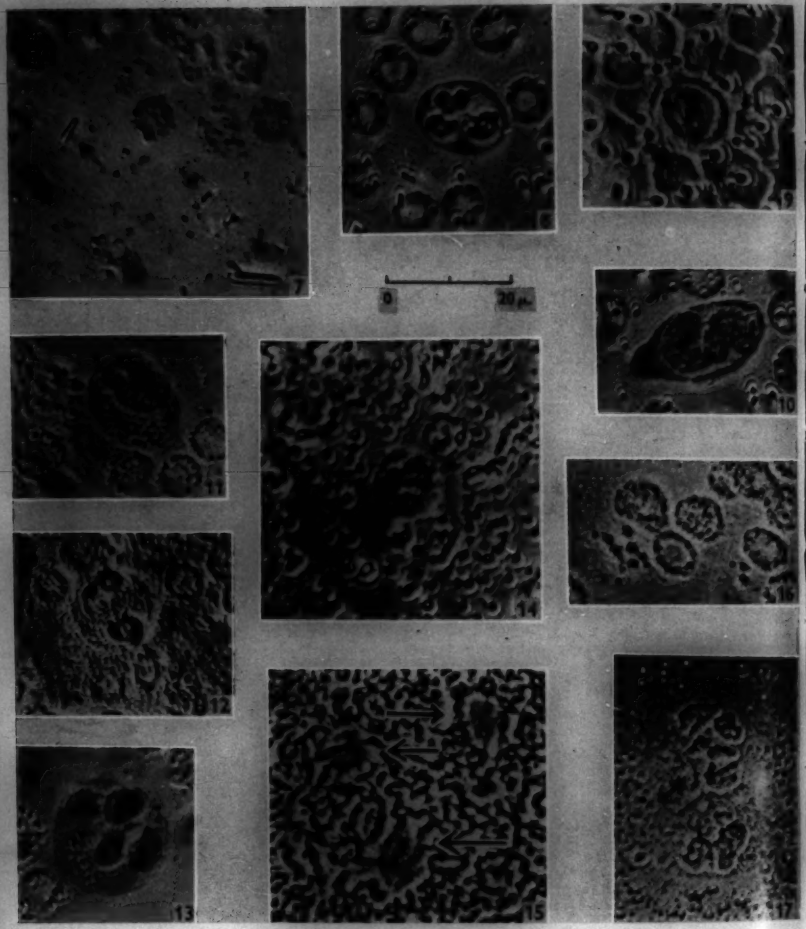

Figs. 7–17.

EXPLANATION OF PLATES

Plate 1

Unless otherwise stated, all figures are phase-contrast microscope photomicrographs.

Fig. 1. Incinerated film showing the appearance of the ash of erythrocytes. The ash of some cells is slightly hydrated and minute droplets appear as black dots. Note that some cells appear darker than others, which suggests that the quantity of ash varies from cell to cell. Compare with Fig. 4.

Fig. 2. Similar, after being breathed upon. Note that some cells are hydrolysed; they appear granular and contain small droplets.

Fig. 3. Similar, after washing in distilled H_2O. Note that some cells now possess but little ash.

Fig. 4. Leitz Ultropak, oil immers. ×50. Incinerated normal erythrocytes, hydrolysed. The resulting droplets are brilliantly refractile and show up as brightly reflecting dots. Note that some cells contain many droplets whilst others contain fewer; and that, with this form of incident illumination, only the droplets are visible.

Fig. 5. Incinerated erythrocytes repeatedly breathed upon and dried, to show crystals of Na and K salts.

Fig. 6. Incinerated erythrocytes, washed in dilute acetic acid to remove Na and K salts; wet specimen. Mineral residues remain, in which Fe was detected.

Plate 2

All figures are phase-contrast microscope photomicrographs.

Fig. 7. Incinerated erythrocytes, washed with dilute acetic acid and dried; a droplet of alcoholic H_2SO_4 was then applied. Arrows indicate the resulting $CaSO_4$ crystals.

Fig. 8. Incinerated blood film. Neutrophil leucocyte. Note halo around nucleus. Surrounding erythrocytes contain droplets.

Fig. 9. Similar, showing small lymphocyte.

Fig. 10. Similar, showing monocyte.

Fig. 11. Incinerated leukaemic blood, showing eosinophil myelocyte. Note nucleus, cytoplasm and granules.

Fig. 12. Incinerated normal blood. Eosinophil leukocyte, showing bilobed nucleus and granules in protoplasm.

Fig. 13. Incinerated leukaemic blood, showing abnormal eosinophil leucocyte with diminished number of granules.

Fig. 14. Incinerated normal blood. Hygroscopic ash of erythrocytes completely hydrated so that outlines of cells are obscured by droplets. In centre a neutrophil polymorph is free from droplets.

Fig. 15. Similar, showing two lymphocytes and a monocyte.

Fig. 16. Incinerated leukaemic blood, showing blood platelets. These seem to consist in part of hygroscopic (darker) and non-hygroscopic (lighter) material.

Fig. 17. Incinerated pus, showing two polymorphs with ingested bacteria. That above contains an obvious chain of cocci.

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SYNTHESIS OF ACETYLCHOLINE IN THE WALL OF THE DIGESTIVE TRACT

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Acetylcholine is continuously released from the wall of the stomach and intestine, and this release occurs independently of the activity of their extrinsic nerves. Until recently it has been the general inclination to attribute this acetylcholine metabolism to the nervous structures of the myenteric and submucous plexus. But the recent finding that the release of acetylcholine continues even after these structures have been paralysed by either curare or cocaine throws serious doubts on this conception. It is possible that the acetylcholine metabolism in the wall of the digestive tract is, to a great extent at least, non-nervous in origin, comparable to that in the human placenta and intimately linked with the choline and lipin metabolism in this tissue (Feldberg & Lin, 1949*a*).

Another view generally held is that the released acetylcholine exhibits only motor functions in the intestine. One can easily trace this view back to the early findings of Le Heux (1918–19), and Magnus (1920) of a continuous release of choline from the intestinal wall and to their conclusion that choline is the hormone of intestinal movements. This view was transferred to acetylcholine when this substance also was found to be continuously released from the intestinal wall. On the other hand, Wright, Jennings, Florey & Lium (1940) and Florey, Wright & Jennings (1941) considered the possibility that the continuous release of acetylcholine might provide a stimulus for the continuous secretion of succus entericus. Their view was based on the findings that the secretion is not only abolished by atropine but enhanced by small amounts of eserine.

In the present paper an examination has been made of the distribution of choline acetylase, the enzyme responsible for the synthesis of acetylcholine, along the length of the wall of the small intestine and in its different layers.

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A few experiments were carried out on the stomach and the different parts of the large intestine. From an accurate knowledge of such a distribution it should be possible to obtain information on both problems, that of the origin and that of the function of the acetylcholine metabolism of the wall of the digestive tract.

METHODS

Guinea-pigs, rabbits, cats, kittens, dogs and an old male monkey (*Macacus rhesus*) of 11·4 kg. were used. The guinea-pigs, rabbits and kittens were stunned by a blow on the neck, the head cut off and the trunk allowed to bleed from the severed carotid arteries. The cats and dogs were bled during ether-chloroform anaesthesia, the thorax was opened and the heart cut out. Immediately afterwards the small intestine and any other organ required were removed. In the experiment on the monkey the small intestine was removed without previous bleeding from the living animal under nembutal anaesthesia. The lumen of the intestine was washed out with tap water and the intestine afterwards kept in cold saline solution. In order to remove the mucus often adherent to the mucosa, pieces of intestine were slit open and the inner surface carefully cleaned by gently rubbing over it with a finger, keeping the preparation in ice-saline solution which was replaced several times during the cleaning. The necessity for this procedure became clear in the course of the experiments.

In cats and dogs, five layers of the small intestine were separated. Starting from the lumen they will be referred to as: (1) glandularis mucosa or gl. mucosa, (2) muscularis mucosa or m. mucosa, (3) submucosa, (4) circular muscle layer, and (5) longitudinal muscle layer (with serosa attached to it).

The first two layers represent the mucosa; its m. mucosa consists in these animals of a thick sheath of smooth muscle fibres. The term gl. mucosa refers to that part which is easily scraped off from the m. mucosa with a knife. The scraped-off tissue consisted mainly of the glandular tissue. Usually the scraping off was done from a fresh piece of intestine superficially dried between filter papers and the scraped-off material at once brought into ice-cold acetone. In a few experiments the whole piece of intestine was first immersed in ice-cold acetone, left there for a few minutes and the gl. mucosa then scraped off in the acetone.

The submucosa consists, in cats and dogs, of a tough membranous sheath of connective tissue which is easily removed *in toto*. Its dry weight amounts to about 15–20% of that of the whole wall.

In order to separate the two layers of muscularis externa the piece of intestine was often slipped over a glass rod before the outer longitudinal muscle was stripped off with a needle according to the method described by Magnus (1920). The serosa was not removed and is therefore included in the layer termed longitudinal muscle.

The pieces of intestine or its respective layers were cut into fine pieces in ice-cold acetone and then ground in a mortar for final maceration with fresh acetone. The acetone was filtered off by suction. From the dried material saline extracts were prepared using 1·5 ml. for each 50 mg. dried tissue. The saline extracts were centrifuged and the supernatant fluid only used for incubation. The procedure for setting up a sample was as follows: 1·5 ml. saline extract, corresponding to 50 mg. dried tissue, were incubated for 1 hr. in a volume of 4·5 ml. The remaining 3 ml. consisted of 1·5 ml. of saline solution and 1·5 ml. of a solution containing the following constituents: 3 mg. choline, 4·5 mg. cysteine (solution made up immediately before use), 0·4 mg. ATP–P (corresponding to 8 mg. of the Ba–ATP which is converted to the sodium salt before use), 12 mg. Na-citrate, 3 mg. NaF, 6 mg. KCl, 4 mg. $MgCl_2$, 0·5 mg. eserine sulphate, 0·3 ml. standard phosphate buffer solution (pH 7–7·2). This method has been previously described (Feldberg & Mann, 1946).

For optimal synthesis of acetylcholine sufficient activator or coenzyme A must be present in the synthesizing medium. Samples were therefore frequently set up with additional activator prepared from acetone-dried tissue of rabbit or guinea-pig brain by extraction with saline solution (1·5 ml./50 mg. dried tissue), centrifuging the extract and boiling the supernatant fluid containing the activator. After filtration and cooling 1·5 ml. of the boiled extract were added to each sample,

instead of the 1·5 ml. of saline solution. The term activator, and not coenzyme A, is used throughout this paper because no purification was carried out.

In some experiments the acetylcholine content of the fresh intestinal tissue was determined. For this purpose the acetylcholine was extracted with HCl and then boiled. For each g. tissue about 2 ml. 1/3 N-HCl and 1 ml. phosphate buffer solution containing 0·1 mg. eserine sulphate were used. The tissue was ground with some sand in this mixture and then boiled for a short time. Before being assayed for acetylcholine it was cooled and neutralized.

The acetylcholine assay was carried out on the eserinized frog rectus muscle with the precautions necessary to eliminate the effects of substances present in the extracts which sensitize the frog rectus muscle to acetylcholine (Feldberg & Mann, 1945–6).

RESULTS

Choline acetylase was found to be present in the wall of the small intestine and in all its layers with the exception of the membranous submucosa. In the course of the experiments it soon became clear that the values obtained for some tissues might be too low on account of lack of activator or of presence of an inhibitor.

Lack of activator. This was responsible for the low values obtained with extracts of the muscularis externa, the longitudinal as well as the circular muscle layer. These extracts synthesized often two to three times more acetylcholine when additional activator had been added to the synthesizing medium. With extracts of the gl. and m. mucosa, the effect of additional activator was usually less pronounced. With extracts prepared from the whole wall the results varied according to species. Synthesis by extracts of rabbit's intestine was little if at all affected, whereas the synthesis by extracts of the wall of the small intestine of the other species increased somewhat by additional activator. This is seen from the results given in Table 1. In later experiments, if not otherwise stated, additional activator was therefore added to all samples.

Presence of an inhibitor. Hsu & Chang (1943) mention the presence of an inhibitor for the synthesis of acetylcholine in the fresh mucosa of the dog's small intestine. We found that the inhibitor is also present in extracts of the acetone-dried tissue but only of the gl. mucosa. This was seen when the extracts of acetone-dried mucosa of the dog's small intestine were examined separately and together. Gl. mucosa alone synthesized 2 μg./g., and m. mucosa alone 65 μg./g.; but m. mucosa synthesized only 2·5 μg./g. when incubated with an equal amount of gl. mucosa. Next the inhibiting effect of an extract of gl. mucosa was examined on extract of rabbit brain which synthesized 1025 μg./g./hr. acetylcholine. In the presence of an equal amount of gl. mucosa in the incubation medium this value was reduced to 105 μg./g./hr.

No inhibition was produced by the extracts from acetone-dried m. mucosa or muscularis externa. The inhibition observed with extracts from the whole wall must therefore originate from the gl. mucosa. In rabbits the inhibitor was found along the whole length of the small intestine, in guinea-pigs in the duodenum and jejunum only. The following figures are mean values of three

experiments (Exps. 6, 7 and 8 of Fig. 1) with guinea-pig's intestine. The saline extracts of the acetone-dried tissue of duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum synthesized 193, 120, 130, 175 and 220 μg./g./hr. acetylcholine respectively. These extracts were, in addition, incubated together with extract of acetone-dried brain. Brain (1 g.) plus intestinal tissue (1 g.) synthesized 610, 780, 890, 1060 and 1030 μg. acetylcholine. If we subtract from

Table 1. Effect of additional activator on synthesis of acetylcholine in extracts from the wall of the small intestine. Figures represent μg. acetylcholine synthesized per g. acetone-dried tissue in 1 hr. without (*a*) and with (*b*) additional activator

	Guinea-pig		Rabbit		Monkey		Dog		Cat		Kitten	
Layers	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
Whole wall	170	200	73	103	20	35	80	125	35	50	40	48
	350	410	80	90	75	113					35	50
			100	95								
			100	97								
			100	110								
			113	125								
			126	125								
			126	150								
			133	155								
Muscularis externa	—		—		14	37	50	70	—		—	
					35	95						
					30	60						
Longitudinal muscle	—		—		—		23	60	15	32	—	
							25	53	25	35		
							45	100				
Circular muscle	—		—		—		3	15	20	35	—	
							9	18	13	25		
							27	56				
Mucosa	—		—		—		—		—		31	25
M. mucosa	—		—		4	13	68	70	13	18		
							80	120	20	15		
									10	14		
Gl. mucosa	—		—		200	230	42	78	20	13		
							55	80	25	35		
							115	160	25	30		
							100	130				

these values those for the synthesis of the respective intestinal tissues we would obtain per g. brain alone a synthesis of 417, 660, 760, 850 and 840 μg. respectively. These figures have to be compared with 850 μg./g., the amount synthesized on incubation of brain extract alone. Thus only the extracts from the ileum did not inhibit the synthesis in brain extracts. In the presence of extract from duodenum, upper and lower jejunum it was depressed by 51, 28 and 11% respectively. In a similar single experiment with rabbit intestine (Exp. 6, fig. 2) the inhibition produced by the five corresponding extracts of intestinal tissue amounted to 55, 70, 55, 40 and 70 %.

The inhibitor was found to be heat sensitive. Extracts brought to boiling-point lost their inhibiting effect. For instance an extract from dog's gl. mucosa which reduced the synthesis of acetylcholine in rabbit's brain extract from

600 to 260 μg./g. increased it to 700 μg./g. after it had been boiled. The increase results from the presence of activator in gl. mucosa extracts, which only becomes evident after the inhibitor has been destroyed.

The strong heat-sensitive inhibitor would naturally interfere with the determination of choline acetylase activity in extracts of gl. mucosa, but it is possible to account for this factor by determining the inhibition produced by extracts of gl. mucosa on the synthesis of acetylcholine in brain extracts. For instance, in the previously mentioned experiment duodenal extract synthesized 193 μg./g. acetylcholine, but inhibited its synthesis in brain extract by 51%. Assuming a similar inhibition to have occurred in the duodenum extract itself the value of 193 μg./g. would represent about half the actual enzyme activity. It is not certain, however, if we are really justified in making this assumption.

Table 2. Inhibition by extracts of glandularis mucosa of the synthesis of acetylcholine in brain extracts

Animal and region of gl. mucosa	μg. acetylcholine synthesized in 1 hr. at 37° C. in extract from: 1 g. dried intestine	1 g. dried brain	1 g. dried intestine plus 1 g. dried brain	Percentage inhibition (−) or percentage augmentation (+)
Dog (duodenum)	90	940	840	−20
	115	940	880	−19
	145	1000	560	−58
	57	320	440	+13
	146	900	1050	0
	80	900	1050	+ 8
	60	670	1000	+40
	130	1300	1500	+ 5
Dog (jejunum)	55	940	1000	0
	55	940	950	− 5
	105	1000	770	−33
	37	320	460	+33
	120	900	1020	0
Dog (ileum)	50	940	770	−23
	110	940	880	−18
	125	1000	1070	− 5
	32	320	400	+15
	180	900	980	−11
Cat (duodenum)	35	1000	680	−35
Cat (ileum)	30	1000	850	−18

Fortunately, the inhibitor is not derived mainly from the tissue of the gl. mucosa, but from its secretion, part of which adheres to the mucosa in the form of mucus. When the surface of the mucosa is carefully cleaned so that the attached mucus is removed as completely as possible, the extracts of the acetone-dried gl. mucosa were found no longer to contain the inhibitor, or contained it in small amounts only which usually did not interfere greatly with the determination of choline acetylase activity. For instance, in only five of the twenty experiments of Table 2 was the depression of synthesis in brain extracts greater than 20% when they were incubated with extracts of cleaned mucosa; in nine of the twenty experiments there was either no effect or an augmentation.

This augmentation is again explained by the presence of activator in gl. mucosa extracts.

It is thus possible to assess the synthesizing power of gl. mucosa when the necessary precautions are taken. This has been done in all later experiments. If appreciable amounts of inhibitor are, nevertheless, found in the extracts the values for the synthesis of acetylcholine have either to be discarded or the required corrections to be made.

A heat-sensitive inhibitor of the synthesis of acetylcholine has previously been described by Comline (1946) in acetone-dried spleen tissue. He assumes that the inhibitor is an enzyme which destroys the activator or the co-enzyme. As the inhibitor of the gl. mucosa is present in its secretion and heat sensitive it might also be an enzyme which could destroy the co-enzyme as well as the choline acetylase.

Species differences. The highest values were obtained with extract from the tissue of the guinea-pig's small intestine. In some experiments nearly 500 μg./g./hr. acetylcholine were formed. The usual values were between 150 and 300 μg./g./hr.; in a few experiments the values were even lower. With dog and rabbit small intestine the values were between 60 and 140 μg./g./hr. The intestinal wall of the one monkey examined synthesized in the ileum 135 and in the jejunum 35 μg./g./hr. acetylcholine. Both these extracts contained some inhibitor and in about equal amounts. Extracts from the small intestine of the few cats and kittens examined synthesized between 15 and 50 μg./g./hr. There was practically no inhibition of synthesis with the cat's extracts, but those of the kitten intestine inhibited the synthesis in brain extract by 15–35%.

TABLE 3. Comparison of content of acetylcholine and of choline-acetylase in the wall of the small intestine of different species

Species	μg. acetylcholine synthesized in 1 hr. per g. acetone-dried tissue	μg. acetylcholine contained in 1 g. fresh tissue
Cats	15–50	1·2–3·0
8-day kitten	48	8·0
11-day kitten	27	10·4
17-day kitten	32	7·1
Monkey (jejunum)	35	5·2
Monkey (ileum)	130	11·0
Dog	60–140	1·4–3·0
Rabbit	60–140	2·3–2·6
Guinea-pig	150–480	6–10

Comparison between content and synthesis of acetylcholine. The acetylcholine content is not always a reliable measure for the acetylcholine metabolism in the intestinal wall as seen from the results of Table 3. For instance, the results obtained with kitten intestine show that a high acetylcholine content may be associated with a low concentration of choline acetylase. On the other hand, a low content apparently can be taken as a sign of a low acetylcholine metabolism in the wall of the digestive tract.

Distribution of choline acetylase along the length of the small intestine in guinea-pigs and rabbits

The existence of a gradient in the concentration of choline acetylase in the wall of the small intestine would be of interest in connexion with the theory of Alvarez of a 'metabolic gradient' responsible for the unidirectional movements of the intestinal peristaltic waves. In a number of papers Alvarez and his co-workers (see Alvarez, 1940) claim to have found evidence for a metabolic gradient in the small intestine, but its existence is anything but proved. Since tone and movements of the muscle layers are probably under the influence of the continuously released acetylcholine, a relation of such a gradient with the acetylcholine metabolism would appear plausible. However, no such gradient was found for the choline-acetylase distribution along the length of the small intestine.

The guinea-pig small intestine has a thin wall and is usually less than 150 cm. long. The whole length has therefore frequently been used and cut into pieces of 20–30 cm. length which were dried separately with acetone. The rabbit small intestine is longer and its wall is thicker, so that sections from different regions only have been used. In Figs. 1 and 2 the distribution of choline-acetylase along the length of the small intestine is plotted. In order to obtain comparable curves the variations in length of the individual intestines are eliminated, the abscissae indicate the length of each piece of intestine and its distance from the pylorus not in cm. but in percentage of the total length of the small intestine. The actual length in cm. is given, however, for each curve. Similarly, in order to eliminate the variations in the synthesizing power of the individual intestines the ordinates refer to the synthesis as percentages of the highest values obtained for each experiment and not to the μg./g./hr. acetylcholine synthesized; but again the highest value for each experiment is given in this form.

In seven of the nine experiments of Fig. 1 with guinea-pig intestine the distribution curve of choline-acetylase shows a trough in the region of the jejunum; the maximal variations in these experiments amount to 51, 45, 51, 52, 51, 63 and 32% respectively. In other words, the tissue from both ends of the small intestine synthesized about twice as much acetylcholine as that obtained from the jejunum. Exps. 5 and 9 do not follow this pattern, but in Exp. 5 it is at least discernible.

The observed variations are greater than those inherent in the method. For instance, one guinea-pig small intestine was divided into three equal pieces which were put together end to end and cut again into six sections, each of which was dried separately with acetone. Extracts from these samples synthesized 285, 326, 330, 330 and 330 μg./hr./g. dried tissue of acetylcholine respectively. In another experiment the guinea-pig small intestine was cut

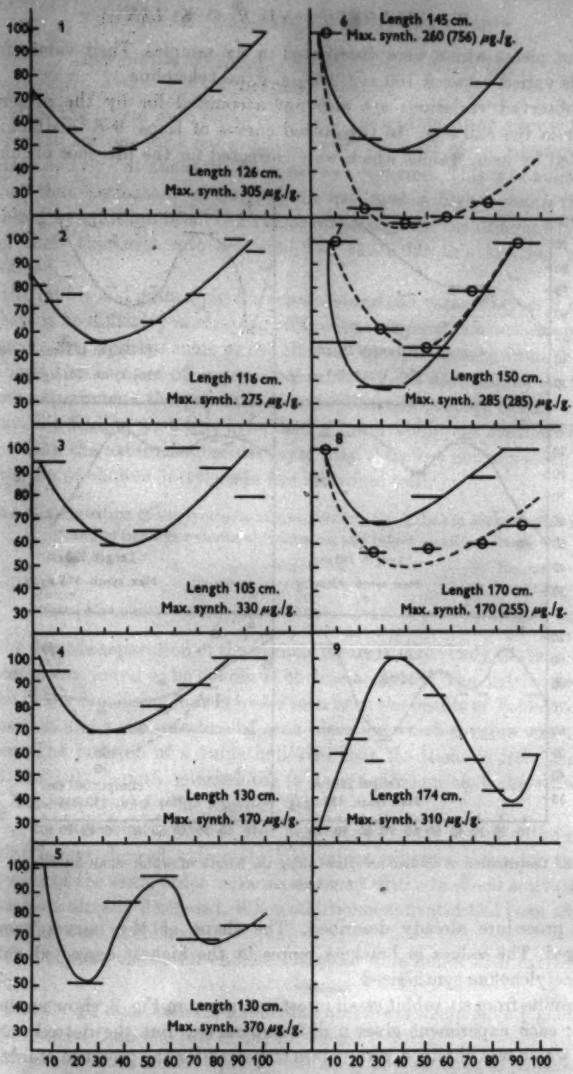

Fig. 1. Distribution of choline-acetylase along the length of guinea-pig small intestine. For details see text.

into sixty pieces which were distributed in six samples. Their values for the synthesis varied between 160 and 200 μg./g. acetylcholine.

The observed variations are also not accounted for by the presence of inhibitor in the extracts. In the dotted curves of Exps. 6–8 this factor was eliminated by using values which were corrected for the presence of inhibitor

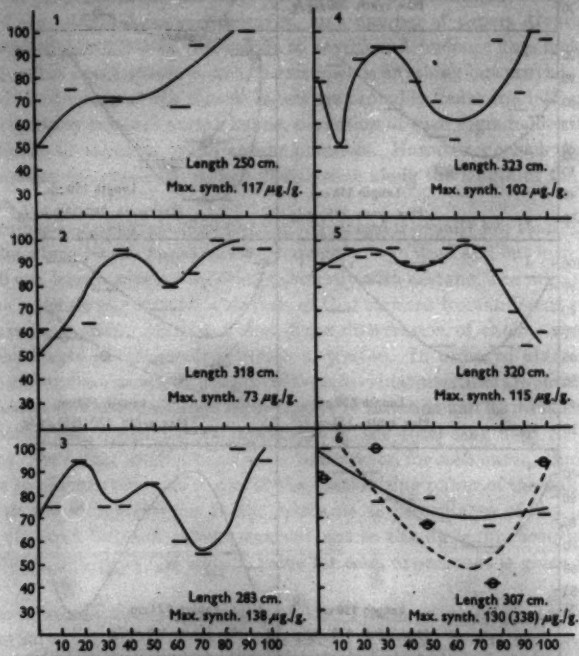

Fig. 2. Distribution of choline-acetylase along the length of rabbit small intestine. For details see text.

by the procedure already described. The shape of the curves remained unchanged. The values in brackets represent the highest corrected value in μg./g. acetylcholine synthesized.

The results from six rabbit small intestines, given in Fig. 2, show no uniform pattern; each experiment gives a different curve. But the dotted curve of Exp. 6, which was obtained with values corrected for the presence of inhibitor, shows a trough which is situated nearer the ileum than in the corresponding experiments with guinea-pig intestine.

Distribution of choline-acetylase in the different layers of the small intestine

Whether the layers were removed from a piece of intestine after it had been immersed in acetone for a few minutes, or from a fresh piece, a loss of choline-acetylase activity of about 10–20% always occurred. This was evident from the fact that extraction of the wall *in toto* gave a higher value than that calculated by addition of all the values obtained with the different layers and taking their thickness into account. The cause for this loss has not been ascertained.

In the rabbit and guinea-pig the separation of the various layers is not easy, but there is no difficulty in scraping off the inner layer which consists mainly of gl. mucosa and perhaps some of the thin m. mucosa. As seen from the results of Table 4 this scraped-off inner layer synthesizes acetylcholine. At the time of these experiments the presence of an inhibitor in mucosa extracts was not known. The finding that this layer had a lower ability to synthesize acetylcholine than the external muscular layer may therefore not represent the true distribution of choline-acetylase in the intestinal wall.

TABLE 4. Comparison of the synthesis of acetylcholine (μg./g./hr.) in acetone-dried mucosa and muscularis externa of guinea-pig and rabbit small intestine

	Guinea-pig					Rabbit		
Mucosa	75	80	160	180	210	20	21	52
Muscularis externa	100	110	370	360	480	50	80	55

In the dog the separation of the various layers is easy. Only the membranous submucosa was found to be devoid of choline-acetylase. The distribution of the enzyme in the remaining four layers is seen from the results of Table 5, in which the position along the intestine of each piece for which a value was obtained is given. The position of a value indicates that the tissue is from the region within the 10% length referred to; it does not mean that the whole 10% length has been used for the sample.

When no additional activator had been added to the samples, the external muscular layers showed extremely low values, whereas those for the other layers were of the same order as those obtained with additional activator. This fact has been already discussed. When additional activator had been added the main results were as follows:

(1) The highest values for choline-acetylase were found with extracts from gl. mucosa.

(2) Usually gl. mucosa from duodenum and lower ileum gave higher values than gl. mucosa from the rest of the intestine.

(3) M. mucosa synthesized per g. less acetylcholine than gl. mucosa from the same piece of intestine but as much as, and sometimes even more than, the two layers of the muscularis externa.

TABLE 5. Synthesis of acetylcholine (μg./g./hr.) in layers of dog small intestine. (G = gl. mucosa; M = m. mucosa; C = circular muscle layer; L = longitudinal muscle layer.)

		Length in percentage of small intestine									
		Duodenum									Ileum
Exp. no.	Layer	1–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100
1	G	105	—	55	—	70	—	—	70	50	110
	M	20	—	35	—	40	—	—	63	33	70
	C	17	—	27	—	30	—	—	36	45	17
	L	83	—	65	—	48	—	—	80	65	80
2	G	145	—	—	—	—	87	—	—	—	125
	M	70	—	—	—	—	65	—	—	—	90
	C	87	—	—	—	—	55	—	—	40	40
	L	75	60	40	65	45	40	70	73	65	60
3	G	220	—	—	—	—	—	—	—	—	—
	M	75	—	—	—	—	—	—	—	—	—
	C	75	—	—	—	—	—	—	—	—	—
	L	120	85	80	90	70	—	—	80	105	105
4	G	80	—	—	120	—	—	—	—	180	—
	M	68	—	—	75	—	—	—	—	85	—
	C	80	—	—	70	—	—	—	—	100	—
	L	70	—	—	110	—	—	—	—	23	—
5	G	145	—	—	—	—	—	—	—	—	—
	M	65	—	—	—	—	—	—	—	—	—
	C	110	—	—	—	—	—	—	—	—	—
	L	42	—	—	—	—	—	—	—	—	—
6	G	60	—	—	—	—	—	—	—	—	—
	M	45	—	—	—	—	—	—	—	—	—
	C	30	—	—	—	—	—	—	—	—	—
	L	50	—	—	—	—	—	—	—	—	—
7	G	130	—	—	—	—	—	—	—	—	—
	M	120	—	—	—	—	—	—	—	—	—
	C+L	70	—	—	—	—	—	—	—	—	—
8	C	30	—	—	15	—	—	—	25	—	—
	L	95	—	—	53	—	—	—	50	—	—
9*	G	100	60	50	—	—	—	26	70	—	60
	M	62	45	52	—	—	—	35	53	—	53
	C	15	18	—	—	9	—	—	—	—	—
	L	8	8	—	—	10	—	—	—	—	—
10*	G	140	115	110	—	130	—	75	85	55	65
	M	80	68	95	—	120	—	140	95	115	90
11*	M	80	67	62	95	125	—	130	—	118	80
12*	M	50	38	35	48	—	—	42	—	35	52

* Without additional activator in the samples.

(4) The choline-acetylase of the muscularis externa was not confined to, and often not particularly concentrated in, the longitudinal muscle layer to which the nerve cells of the myenteric plexus are said to adhere when this layer is stripped off. In fourteen of nineteen experiments in which the two layers of the muscularis externa were examined separately from the same piece of intestine the longitudinal muscle synthesized per g. more acetylcholine than the circular muscle but in the other five experiments the reverse was true.

The table shows also that the individual variations of the values are so great that with the exception of the high concentration at the end parts of the gl.

mucosa there are no characteristic and regular differences between the various layers or the various regions of the small intestine. This is seen perhaps even more strikingly in Table 6, where the results are arranged in three columns, the two end parts and the rest of the small intestine, and where only the mean values are given. They are calculated from those intestinal pieces of Exps. 1–6 of Table 5 for which all four layers have been examined.

TABLE 6. Synthesis of acetylcholine (μg./g./hr.) in layers of small intestine of the dog. Mean values from Exps. 1 to 6 of Table 4

(G, M, C and L as in Table 4)

	Regions in percentage of length of small intestine		
Layers	Duodenum (1–10)	Jejunum-ileum (10–80)	Lower ileum (80–100)
G	125	60	114
M	57	56	70
C	67	43	50
L	73	49	57

TABLE 7. Contribution in mg. by the different layers to 1 g. of wall of the dog's small intestine

	Weight of layer in mg./g. wall			
Layers	Duodenum	Jejunum	Ileum	Lower ileum
Gl. mucosa	250	266	223	196
M. mucosa	425	318	283	262
Mucosa	675	584	506	458
Circular muscle	226	315	374	381
Longitudinal muscle	99	101	120	161
Muscularis externa	325	416	494	542

Since the thickness of the layers varies along the length of the intestine the foregoing results do not indicate the contribution each layer makes to the acetylcholine synthesizing power of the whole wall. In Table 7 the acetone-dried weight of each layer is given in mg./g. of wall which, however, does not include the membranous submucosa. It will be seen that the longitudinal muscle layer contributes least to the wall, although its thickness increases somewhat from duodenum to ileum; that the circular muscle is two to three times thicker than the longitudinal muscle; that the gl. and m. mucosa make up about two-thirds of the thickness of the wall in the duodenum but less than half in the ileum; and that this change is mainly accounted for by variations in the thickness of the m. mucosa. From the same samples which were used for calculation of Table 7, the mean values are given in Table 8 for the synthesis of acetylcholine. This enables us to assess the contribution of each layer to the acetylcholine synthesizing power of the whole wall.

TABLE 8. Distribution of choline acetylase in layers of dog small intestine

Layers	μg. acetylcholine synthesized per hr. per g. layer				μg. acetylcholine synthesized per hr. in layer per g. wall				Percentage distribution of choline acetylase in wall			
	D	J	I	LI	D	J	I	LI	D	J	I	LI
Gl. mucosa	112	98	98	118	28·0	26·1	21·9	23·1	30	38	32	32
M. mucosa	53	59	57	80	22·5	18·8	16·1	21·0	31	28	23	28
Mucosa					50·5	44·9	38·0	44·1	70	66	55	60
Circular muscle	61	52	66	29	13·8	16·4	24·7	23·1	19	24	36	15
Longitudinal muscle	76	76	50	80	7·5	7·0	6·0	21·0	11	10	9	25
Muscularis externa					21·3	23·4	30·7	44·1	30	34	45	40
Whole wall					71·8	68·3	68·7	74·0				

(D = duodenum; J = jejunum; I = ileum; LI = lower ileum.)

The mucosa contributes about 70% in the duodenum and about 60% in the ileum to the choline-acetylase content of the wall. The gl. mucosa contributes slightly more than the m. mucosa and contains usually more choline-acetylase than any other layer, i.e. between 32 and 39% of the total amount although it represents only 20–25% of the tissue of the wall. The longitudinal muscle layer, despite its occasionally high choline-acetylase concentration, contributes least, i.e. only between 9 and 25%. A higher contribution, between 15 and 36%, is made by the much thicker circular muscle layer, although its choline-acetylase concentration is often lower than that of the longitudinal muscle layer.

In Exp. 7 of Table 5 the muscularis externa was not separated into its two layers. Leaving the membranous submucosa again out of the calculation. 1 g. acetone-dried wall consisted of 31% muscularis externa, 35% gl. mucosa and 34% m. mucosa. One gram wall synthesized per hr. 108 μg. acetylcholine, of which 21·7 μg. came from the muscularis externa, 40·8 from the m. mucosa and 45·5 μg. from the gl. mucosa. The mucosa thus contributed as much as 80% to the synthesizing power of the wall.

Two experiments on cats were carried out (see Table 9). Although the choline-acetylase concentration in the whole wall is much lower than in the dog's intestine its distribution in the different layers shows the same trend. Again, the two layers of the mucosa contribute between 60 and 70% to the choline-acetylase content of the wall and the longitudinal muscle layer contributes least. This muscle in cats is about six to seven times thinner than the circular muscle, so that even in the first experiment in which the longitudinal muscle synthesizes per g. tissue over six times as much acetylcholine as the circular muscle, its contribution is less than that of the circular muscle layer.

In the two kittens examined the mucosa and muscularis externa, as far as these layers could be separated by scraping off the inner layer, synthesized about the same amounts of acetylcholine per g. acetone-dried tissue. The scraped-off mucosa made up between 30 and 40% of the total wall.

TABLE 9. Synthesis of acetylcholine in layers of cat small intestine

Exp. and region	Layer	Percentage contribution of layer to wall	μg. acetylcholine synthesized in 1 hr.	
			Per g. layer	Per g. wall
Exp. 1 (jejunum)	Gl. mucosa	18	20	3·6
	M. mucosa	24	18	4·3
	Circular muscle	51	5	2·6
	Longitudinal muscle	7	32	2·2
Exp. 2 (duodenum)	Gl. mucosa	30	35	10·5
	M. mucosa	36	20	7·2
	Circular muscle	31	35	10·9
	Longitudinal muscle	3	35	1·1
Exp. 3 (ileum)	Gl. mucosa	32	30	9·6
	M. mucosa	27	13	3·5
	Muscularis externa	41	14	5·7

In the monkey the different layers were examined in two pieces of ileum using slightly different procedures for each piece. From the one both layers of mucosa were scraped together in acetone and dried separately from the remaining tissue (submucosa *plus* muscularis externa). From the other piece the gl. mucosa was scraped off and dried separately before the preparation was placed in acetone. The m. mucosa was then scraped off and also dried separately. The results, which are given in Table 10, show that the mucosa contained about 80% of the choline-acetylase of the wall, and that it was mainly localized in the gl. mucosa in which the concentration reaches the highest level.

TABLE 10. Synthesis of acetylcholine in layers of monkey ileum

Layer	Percentage contribution of layer to wall	μg. acetylcholine synthesized in 1 hr.			
		Per g. layer		Per g. wall	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Mucosa	66	125	—	82·5	—
Gl. mucosa	35	—	230	—	80·5
M. mucosa	31	—	13	—	4·0
M. externa	34	65	37	22·1	12·6

Distribution of choline-acetylase in other tissues

In the rabbit no choline-acetylase was detected in the acetone-dried tissue of the oesophagus, the thin wall of the caecum or the urinary bladder.

In the guinea-pig the enzyme was absent in the tissue of the oesophagus and uterus. The one urinary bladder examined synthesized 40 μg./g./hr. acetylcholine as compared to 360 μg./g./hr. (the highest value) for the small intestine in this animal. Low values were obtained for the stomach and caecum, whereas the values for the colon were of the same order as those for the small intestine. Table 11 gives the results of four experiments.

In one experiment pieces from the cardia, fundus and pyloric region of the stomach were examined separately. The inner layer was scraped off in acetone

and dried separately. It consisted mainly of gl. mucosa and m. mucosa, although it was not possible with this method to give a very sharp separation. The outer layer, however, consisted mainly of muscularis externa. The same procedure was adopted for pieces of the duodenum, caecum and colon. As seen from the results given in Table 12, both layers synthesized acetylcholine, and, with the exception of the colon, contained the enzyme in about equal concentrations.

TABLE 11. Synthesis of acetylcholine in different parts of guinea-pig digestive tract

Tissue	μg./g./hr. acetylcholine synthesized in saline extracts of acetone-dried tissue			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Oesophagus	0	0	0	—
Stomach	50	—	29	51
Small intestine (highest value)	360	460	86	—
Caecum	90	—	19	—
Colon	320	310	98	—

TABLE 12. Synthesis of acetylcholine in μg./hr./g. acetone-dried tissue and per square cm. area of wall of guinea-pig digestive tract

	Acetylcholine (μg./hr./g.)		mg. acetone-dried tissue per cm.² wall		Acetylcholine (μg./hr./cm.²)	
	Mucosa	Musc. ext.	Mucosa	Musc. ext.	Mucosa	Musc. ext.
Oesophagus	0	0	5·3	3·2	0	0
Stomach (cardia)	20	16	12·3	2·0	0·246	0·032
Stomach (fundus)	33	27	12·0	2·4	0·396	0·065
Stomach (pyloric)	30	50	10·8	6·1	0·324	0·305
Duodenum	75	110	7·6	3·2	0·570	0·352
Caecum	24	18	2·3	8·6	0·055	0·155
Colon	140	70	2·8	4·3	0·392	0·301

In the colon its concentration in the inner layer, the mucosa, was double that in the outer layer. The synthesis of acetylcholine has also been expressed so as to show the contribution each layer makes to the choline-acetylase content of the whole wall. With the exception of the caecum the mucosa contributes more than the muscularis externa. The greatest differences in the two layers are found in the cardia and fundus of the stomach and in the caecum; they result from the great variation in thickness of the two layers in these regions.

In the dog some choline-acetylase is present in the wall of the oesophagus, more is present in the wall of the stomach and large intestine but not as much as in the wall of the small intestine. The distribution of choline-acetylase in the different layers is shown in Table 13 which gives mean values from three experiments. In assessing these results it has to be realized that it is not possible to separate in the stomach the glandular tissue from the m. mucosa as completely as in the small intestine. The term gl. mucosa refers, with regard to the stomach, only to a superficial layer of mucosa which can be easily scraped off and which in the fundus consisted of 13–20 and in the pyloric region of 25–30% of the whole mucosa. It was also not possible to obtain a clear separation of the longitudinal and circular muscle layer from the stomach and large intestine.

For these regions the terms longitudinal and circular muscle refer to outer and inner parts of the muscularis mucosa only. In spite of these limitations, however, the results of Table 13 show clearly that the wall of the stomach and large intestine synthesize less acetylcholine than does that of the small intestine, and that in the stomach and large intestine, unlike in the small intestine, the enzyme is not concentrated in the glandular layer of the mucosa. In fact, the superficial layer of the stomach mucosa is almost devoid of the enzyme and the gl. mucosa of the large intestine contains small amounts only.

TABLE 13. Synthesis of acetylcholine in layers of dog digestive tract

(G = gl. mucosa; M = m. mucosa; C = circular muscle layer; L = longitudinal muscle layer; W = whole wall.)

	Synthesis of acetylcholine in μg./hr./g. acetone-dried layer								
	G	M	C	L	W	G	M	C	L
Oesophagus	8·5		10		9·4	3·8		5·6	
Stomach (fundus)	3·3	66	63	13	53·0	0*	18	23	12
Stomach (pylorus)	1·5	5	43	55	26·0	0*	2	17	7
Small intestine	112·0	62	70	67	87·0	34	24	22	7
Large intestine	18·0	22	68	39	49·0	3	7	29	10

* Less than 0·3 μg.

Comparison of choline-acetylase concentration and density of nerve cells in the muscularis externa of the guinea-pig digestive tract

When the longitudinal muscle layer is stripped off from the different parts of the digestive tract of a guinea-pig the nerve cells of the myenteric plexus adhere to this layer in the oesophagus and stomach but to the circular muscle layer in the small and large intestine, as shown by Irwin (1931). By means of appropriate sectioning and staining methods Irwin was able to count the number of these nerve cells fairly accurately along the whole length of the alimentary tract. In Table 14 his figures are given and compared with the

TABLE 14. Comparison of acetylcholine synthesis and number of nerve cells per sq.cm. of muscularis externa in the digestive tract of the guinea-pig

	Acetylcholine (μg./hr./cm.²)	Nerve cells per cm.²	Ratios	
			Synthesis	Nerve cells
Oesophagus	0·000	1350	0	0·4
Stomach (cardia)	0·032	3500	1·0	1·0
Stomach (fundus)	0·065	7000	2·0	2·0
Stomach (pyloric)	0·305	18000	9·5	5·1
Duodenum	0·352	10000	11·0	2·9
Ileum	0·440	7500	13·8	2·1
Caecum	0·155	4500	4·8	1·3
Colon	0·304	18000	9·5	5·1

values obtained in the experiment of Table 12 for the acetylcholine synthesis of the muscularis externa. Taking the figures obtained for the cardiac region of

the stomach as 1, the ratios for the synthesis of acetylcholine can be compared with those for the number of nerve cells. This has been done in the last two columns of Table 14. The ratios show striking discrepancies as well as similarities. A doubling of nerve cells in the ileum is associated with a nearly fourteen-fold increase in synthesizing power; a three-fold increase in number of nerve cells in the duodenum with an eleven-fold increase in synthesizing power; and a five-fold increase in nerve cells in the pyloric part of the stomach and in the colon with a nine-and-a-half-fold increase in synthesizing power. On the other hand, there is some parallelism. The synthesizing power and the number of nerve cells increase from oesophagus to the pyloric part of the stomach, whereas the caecum with its relatively low power of synthesis shows on comparison an even lower ratio for the number of nerve cells.

DISCUSSION

Site of acetylcholine metabolism. The distribution of choline-acetylase in the various layers of the intestinal wall provides new evidence in favour of the assumption that the nerve cells of the myenteric and submucous plexus are not the site of the acetylcholine metabolism. In dogs and cats the gl. mucosa yielded very high values for the synthesis of acetylcholine, in fact often higher values than those from any other layer of the intestinal wall; but the gl. mucosa contains no nerve cells, since the cells of the submucous plexus are situated in the submucosa. We do not know if they adhere to the membranous submucosa or to the lower surface of the m. mucosa when this layer is stripped off: if to the submucosa, the absence of choline-acetylase in this layer and its presence in the m. mucosa would be further proof that the enzyme is not located in the nerve cells.

In dogs and cats the cells of the myenteric plexus are said to adhere to the longitudinal muscle when it is stripped off from the circular muscle layer. Dikshit (1938), in fact, had found that slices from longitudinal muscle *plus* serosa synthesized per g. tissue six to ten times as much acetylcholine as slices from circular muscle. He therefore looked upon the cells of the myenteric plexus as the site of the acetylcholine metabolism. With our method of synthesis the differences were smaller. Per g. tissue, synthesis by the longitudinal muscle layer rarely exceeded three times that of the circular muscle; usually the differences were less than 50% and in some experiments circular muscle tissue actually synthesized more than that of the longitudinal muscle. In addition, this layer is much thinner so that, per unit area intestinal wall, it contained usually less choline-acetylase than the circular muscle layer. It is then difficult to see how the cells of the myenteric plexus can be the sole or even the main site of the acetylcholine metabolism in the muscularis externa.

The answer is not so clear with regard to the experiments in which a comparison was made between the number of cells of the myenteric plexus and the

concentration of choline-acetylase per unit area muscularis externa of the guinea-pig intestine. In the stomach both tissue constituents increased to the same extent from cardia to pylorus. There was also a certain agreement in the changes which occurred with the two constituents from small intestine to caecum and colon. They were found to be low in the caecum but high in the colon and small intestine; on a more quantitative basis, however, the parallelism broke down. A doubling in the number of nerve cells was associated in one instance with a two-fold, in another with a fourteen-fold increase in choline-acetylase concentration.

In evaluating these results we must remember, however, that the values for the number of nerve cells and for the content of choline-acetylase were obtained by different authors; the measurements of the areas may therefore not be comparable in the two sets of experiments. Nevertheless, some of the discrepancies were too great to be accounted for in this way. Some correlation between density of nerve cells and concentration of choline-acetylase in the different regions is to be expected, even if the nerve cells are not the site of the acetylcholine metabolism, because both cell constituents are probably associated with motor activity: the nerve cells with the peristaltic reflex, the acetylcholine metabolism with maintenance of tone and rhythmic movements.

Even if the nerve cells are not the site of the acetylcholine metabolism this does not exclude nervous structures in general. The choline-acetylase of cholinergic nerves is, as far as we know, located in the axon and not necessarily in the cell body; therefore regions with an excessive supply of non-myelinated cholinergic nerve fibres should be particularly rich in choline acetylase. Quantitative figures on the number of nerve cells are no precise indication of the density of nerve fibres, although regions rich in nerve cells are probably also rich in non-myelinated nerve fibres. So far as this is the case, the distribution of the choline-acetylase in the various layers does not suggest an intimate association with nerve fibres.

If we assume a non-nervous origin of the acetylcholine metabolism in the intestinal wall it is probably closely linked to the choline metabolism, the choline in turn being derived from the large amounts of lecithin present. On this assumption the high content of choline-acetylase in the gl. mucosa becomes understandable, since this layer must be particularly rich in lipins. There are previous findings which stress the preponderant role of the mucosa in choline and acetylcholine metabolism, although hitherto no distinction has been made between glandular layer and m. mucosa. According to Abderhalden & Paffrath (1925) the choline output from the mucosa of the cat intestine is ten times greater per g. tissue than from the muscularis externa. Feldberg & Solandt (1942) later found that from the perfused rabbit small intestine more choline oozes out into its lumen, that is, from the mucosa, than was obtained from its venous effluent. Dikshit (1938) observed that, per g. tissue, the mucosa of the

cat and dog small intestine synthesized about 50% more acetylcholine than the muscularis externa with serosa attached to it.

An acetylcholine metabolism of non-nervous origin is not confined to the digestive tract. It certainly occurs in the human placenta, has been suggested for the auricle (Abdon, 1945; Burn & Vane, 1949; Bülbring & Burn 1949) and may be responsible for the high content of acetylcholine found in the cornea of several animals (v. Brücke, Hellauer & Umrath, 1949).

If nervous elements are not the main site of the acetylcholine metabolism the problem naturally arises: What are the structures containing the enzyme? The problem is as unsolved as that of the origin of the different hormones in the gastric and intestinal mucosa. The fact that the thin wall of the guinea-pig small intestine is so much richer in choline-acetylase than that of the other animals examined, suggests perhaps that the enzyme is not present in the smooth muscle fibres or in the gland cells. But it is dangerous to speculate about this problem without further experimental evidence. We have to realize that similar species differences are found for the choline-acetylase content in brain (Feldberg & Mann, 1946) and a different proportion of supporting tissue does not appear to be its sole explanation.

Even if the spontaneous release of acetylcholine is not associated with nervous structures some of the choline acetylase in the intestinal wall must be nervous in origin because of its cholinergic parasympathetic nerve supply. The extent of this contribution may vary in the different layers and sections of the intestine. Evidence for a nervous participation is found in the increased output of acetylcholine during vagus stimulation from the stomach (Dale & Feldberg, 1934) and small intestine (Bunting, Meek & Maaske, 1935). A nervous component may also account for the particularly high concentration of choline-acetylase in the gl. mucosa of the duodenum because the parasympathetic innervation to the secretory cells appears to be confined to this part of the intestine. Wright *et al.* (1940) showed that vagus stimulation augments secretion in the duodenum but not in the jejunum and ileum.

Function of acetylcholine metabolism. It is reasonable to conclude that the choline acetylase in the various layers is responsible for the continuous release of acetylcholine which occurs in the wall of the digestive tract. The fact that with the exception of the membranous submucosa all layers of the wall of the small intestine contain the choline acetylase suggests different physiological functions of the enzyme. From its presence alone no specific function could be deducted. We require the supporting evidence of the actions of acetylcholine, eserine and atropine on the different layers of the intestinal wall. A special physiological function, if attributed to the acetylcholine metabolism in the intestinal wall, should be imitated by acetylcholine, intensified by eserine and abolished by atropine. These considerations have to be kept in mind when discussing the function of the acetylcholine metabolism in the layers of the intestinal wall.

In the *muscularis externa* the choline-acetylase is associated with motor activity, but that does not necessarily mean with the peristaltic reflex which is a reaction of the intestinal wall to increased pressure. It is certain, however, that increased motility of the smooth muscles will facilitate the reflex. The acetylcholine continuously released in the muscularis externa presumably acts in this way and thus provides the necessary background of tone and rhythmic activity in the muscle, necessary for initiation of the reflex. This assumption is supported by the fact that acetylcholine as well as eserine, on the isolated preparation, do not produce real peristalsis but increased tone and rhythmic contractions. In our previous experiments (Feldberg & Lin, 1949*b*) on the isolated rabbit intestine, the peristaltic reflex could often only be initiated regularly by the increased pressure when the bath fluid contained a low concentration of eserine, thus allowing a higher acetylcholine concentration to develop in the intestinal wall and thereby providing a more advantageous background of increased tone and rhythmic activity.

In the *muscularis mucosa* the presence of choline-acetylase must also be associated with motor activity. This muscle layer plays apparently a much greater role in the propulsion of stomach and intestinal contents than has formerly been assumed (Forssel, 1923; Cole, 1928; Barclay, 1933; Gordon & Singleton, 1939). If the choline-acetylase in the muscularis mucosa were responsible for a continuous release of acetylcholine in this layer, thereby producing motor activity, acetylcholine and eserine should have stimulating actions on this muscle as they have on the muscularis externa. Recently, Holton (1949) has shown that a preparation of dog small intestine from which the muscularis externa had been stripped off contracts to eserine and acetylcholine.

The presence of choline acetylase in the *glandular mucosa* may be associated with motor and secretory functions and possibly even with absorption. Muscle fibres of the m. mucosa extend into the tips of the villi, and of necessity are extracted together with the gland cells when the gl. mucosa is scraped off and dried in acetone. The following results suggest that the choline-acetylase in the mucosa may be associated with the movements of the villi. An intravenous injection of acetylcholine causes a single quick and tonic contraction of the villi (Beznak, 1936) and eserine injected intravenously or applied locally initiates automatic movements of the villi which continue for a long time (Verzar & v. Kokas, 1927). Atropine, on the other hand, inhibits the movements of the villi (Hambleton, 1914; King & Arnold, 1922). Acetylcholine, as well as eserine, provokes also strong secretion of succus entericus in the small intestine and even desquamation of the epithelium (Verzar & v. Kokas, 1927; Wright *et al.* 1940). These actions are abolished by atropine. It is therefore probable that the choline-acetylase and the continuous release of acetylcholine in the gl. mucosa provides a physiological stimulus for continuous

secretion of succus entericus. In this connexion it is interesting to note that eserine does not produce gastric secretion and that the superficial layers of the gastric mucosa were found to be free from choline acetylase. Its presence in the stomach mucosa, therefore, appears to be solely connected with motor activity of its strong m. mucosa. Wright, Jennings & Florey (1938) were also unable to provoke secretion from the cat colon with eserine which, however, augmented the secretory response to stimulation of the nervi erigentes. Again, relatively little choline-acetylase was found to be present in the gl. mucosa of the dog large intestine. There is thus some parallelism between the effect of eserine and concentration of choline-acetylase in the gl. mucosa in different sections of the digestive tract.

There remains the possibility that the acetylcholine release plays a role also in absorption. According to Fraser (1946) choline facilitates fat absorption, and Beznak (1936) observed that acetylcholine is a strong stimulus for increased lymph flow from the thoracic duct. This effect was attributed to vaso-dilatation in the pre-capillary arteries and capillaries of the villi, and possibly to increased permeability of the capillary wall. Such vascular changes may be required for absorption.

SUMMARY

1. Synthesis of acetylcholine has been studied in saline extracts of acetone-dried tissue of the wall of the digestive tract with the object of obtaining information about the origin and function of the acetylcholine metabolism in this organ.

2. Per g. acetone-dried tissue the wall of the small intestine synthesized the following amounts of acetylcholine in μg./hr.: guinea-pig, 150–480; rabbit and dog, 60–140; cat and kitten, 15–50; monkey, 35–135.

3. A high acetylcholine content of the wall is not necessarily a sign of a great concentration of choline-acetylase. Kitten small intestine contained as much acetylcholine per g. tissue as that of the guinea-pig.

4. With the exception of the membranous submucosa all layers of the wall of the small intestine contained choline-acetylase although in varying concentrations. This was shown in the cat and dog.

5. A heat-sensitive inhibitor for the synthesis of acetylcholine was found in the acetone extracts of mucosa, derived probably from the mucus adherent to the mucosa. When its surface was carefully cleaned before drying the inhibitory effect was small.

6. The layer of the mucosa on top of the muscularis mucosa, the glandular mucosa, usually gave the highest values for synthesis of acetylcholine, particularly the gl. mucosa from the duodenum and lower ileum. This layer is supposed to be free from nerve cells.

7. Per g. acetone-dried tissue muscularis mucosa synthesized less acetylcholine than gl. mucosa but as much as, and sometimes even more, than the two layers of muscularis externa.

8. The choline-acetylase of the muscularis externa was not confined to, and often not particularly concentrated in, the layer of the longitudinal muscle to which the nerve cells of the myenteric plexus are stated to adhere when this layer is stripped from the circular muscle.

9. The concentration of choline-acetylase in the various layers does not indicate the contribution each layer makes to the acetylcholine-synthesizing power of the whole wall because the thickness of the layers differs and varies along the length of the small intestine. If this factor be taken into account, the two layers of mucosa are shown to contribute about 60–80% to the choline-acetylase content of the wall. The gl. mucosa contributed usually more than any other layer, up to 40%. The thin longitudinal muscle layer, despite its occasionally high choline-acetylase concentration, contributed least, between 9 and 25%.

10. No choline-acetylase was detected in rabbit oesophagus, caecum and urinary bladder or in guinea-pig oesophagus and uterus. Low values were found in guinea-pig stomach, caecum and urinary bladder, and in dog oesophagus, stomach and large intestine. The values for guinea-pig colon, on the other hand, were as high as those for its small intestine.

11. Unlike the small intestine, the stomach and large intestine of the dog contain, in the superficial layer of its mucosa, little or no choline-acetylase.

12. A comparison of choline-acetylase concentration and density of nerve cells in the muscularis externa of guinea-pig alimentary tract was made. The concentration of both tissue constituents changes in the same direction in the different sections, but on a more quantitative basis the parallelism breaks down.

13. The distribution of choline-acetylase in the different parts and layers of the wall of the digestive tract provides further evidence in favour of the theory that the enzyme does not originate mainly from the cells or nerve fibres of the myenteric and submucous plexus, but, like the choline-acetylase of human placenta, is non-nervous in origin.

14. The presence of choline-acetylase of non-nervous origin is probably the basis for the continuous release of acetylcholine in the various layers of the wall of the digestive tract. The possible functions of this acetylcholine metabolism are discussed, taking into consideration the known actions of acetylcholine, eserine and atropine on the various layers. It is concluded that the continuous release of acetylcholine in the muscularis externa provides a background of tone and rhythmic movements of the muscles necessary for initiation of the peristaltic reflex by increased pressure from the lumen. In the mucosa the continuous release of acetylcholine also exerts motor functions. It may stimulate the whole sheath of the muscularis mucosa as well as provide a stimulus for movements of villi. In addition, it probably represents a physiological stimulus for continuous secretion of succus entericus. It may possibly also play a role in absorption.

REFERENCES

Abderhalden, E. & Paffrath, H. (1925). *Pflüg. Arch. ges. Physiol.* **207**, 229.
Abdon, N. O. (1945). *Acta Pharmacol. Toxicol.* **1**, 169.
Alvarez, W. C. (1940). *An Introduction to Gastro-Enterology.* N.Y.: Hoeber.
Barclay, A. E. (1933). *The Digestive Tract; a Radiological Study of its Anatomy, Physiology and Pathology.* Cambridge University Press.
Beznak, A. B. L. (1936). *Quart. J. exp. Physiol.* **26**, 253.
Brücke, H. von, Hellauer, H. F. & Umrath, K. (1949). *Int. J. Ophthamologica*, **117**, 19.
Bülbring, E. & Burn, J. H. (1949). *J. Physiol.* **108**, 508.
Bunting, H., Meek, W. I. & Maaske, C. A. (1935). *Amer. J. Physiol.* **114**, 100.
Burn, J. H. & Vane, J. R. (1949). *J. Physiol.* **108**, 104.
Cole, L. C. (1928). *Acta Radiologica*, **9**, 31.
Comline, R. S. (1946). *J. Physiol.* **105**, 6*P*.
Dale, H. H. & Feldberg, W. (1934). *J. Physiol.* **81**, 320.
Dikshit, B. B. (1938). *Quart. J. exp. Physiol.* **28**, 243.
Feldberg, W. & Lin, R. C. Y. (1949*a*). *J. Physiol.* **109**, 475.
Feldberg, W. & Lin, R. C. Y. (1949*b*). *Brit. J. Pharmacol.* **4**, 33.
Feldberg, W. & Mann, T. (1945–6). *J. Physiol.* **104**, 8.
Feldberg, W. & Mann, T. (1946). *J. Physiol.* **104**, 411.
Feldberg, W. & Solandt, O. (1942). *J. Physiol.* **101**, 137.
Florey, H. W., Wright, R. O. & Jennings, M. A. (1941). *Physiol. Rev.* **21**, 36.
Forssel, G. (1923). *Amer. J. Roentgenol.* **10**, 87.
Fraser, A. C. (1946). *Nature, Lond.*, **157**, 414.
Gordon, S. & Singleton, A. C. (1939). *Surgery*, **6**, 697.
Hambleton, B. F. (1914). *Amer. J. Physiol.* **34**, 466.
Holton, P. (1949). Unpublished experiments; personal communication.
Hsu, F. Y. & Chang, C. P. (1943). *Proc. Chin. Physiol. Soc.* (*Chengtu*), **2**, 31.
Irwin, D. A. (1931). *Amer. J. Anat.* **49**, 141.
King, C. E. & Arnold, L. (1922). *Amer. J. Physiol.* **59**, 97.
Le Heux, J. W. (1918–19). *Pflüg. Arch. ges. Physiol.* **173**, 8.
Magnus, R. (1920). *Naturwissenschaften*, **8**, 383.
Verzar, F. & v. Kokas, E. (1927). *Pflüg. Arch. ges. Physiol.* **217**, 397.
Wright, B. D., Jennings, M. A. & Florey, H. W. (1938). *Quart. J. exp. Physiol.* **28**, 207.
Wright, R. D., Jennings, M. A., Florey, H. W. & Lium, R. (1940). *Quart. J. exp. Physiol.* **30**, 72.

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SOME FACTORS INFLUENCING THE PASSAGE OF FLUID THROUGH INTESTINAL LOOPS IN DOGS

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There exists a wealth of clinical and experimental evidence that intestinal motility is stimulated by meals. That this increased activity is in some way co-ordinated with the progress of digestion and absorption would appear to be a reasonable supposition on general grounds; and this is supported by the finding (Cannon, 1904) that, although meals of different composition traverse the small intestine at different rates, digestion and absorption are normally complete by the time the residues of a meal reach the terminal ileum.

Some attention has been paid to the time-relations of the intestinal response to feeding and to the nervous pathways involved (Barcroft & Robinson, 1929; Castleton, 1934; Douglas & Mann, 1939*a*, 1940); but the relationship of the motility observed to the passage of material through the intestine, and the mechanisms involved in the regulation of this, have not been studied to any significant extent. This is perhaps because the methods commonly employed for the study of intestinal motility do not provide information bearing directly on this problem. Since the contents of the upper part of the small intestine are liquid rather than solid, some method of studying the passage of liquid rather than of a solid object such as a balloon or bolus is required; for as Alvarez (1940) has pointed out, the results of experiments using the latter are difficult to interpret in terms of transportation. Relaxation of the intestine results in cessation of movement of a balloon or bolus, but may facilitate the passage of liquid.

The two preparations usually used for the study of intestinal motility in the conscious animal are the 'Biebl loop', in which a portion of the intestine is exteriorized in a skin tube without interruption of the continuity of the intestine; and the 'Thiry-Vella loop' in which a segment of intestine is resected and the ends exteriorized, with restoration of continuity by anastomosis. The first of these is unsuitable for the study of the passage of fluid through the intestine, and, as generally used, provides no definite information regarding the effect of

the movements observed on the contents. The second preparation is deprived of its intrinsic connexions with the rest of the intestine; and although excitation of motility may be transmitted down the intact intestine after meals by intrinsic pathways (Douglas & Mann, 1939*b*), so that the pattern of activity shown by this preparation may be incomplete, the Thiry-Vella loop is otherwise eminently suited to the study of the factors affecting the passage of liquid through the small intestine, provided that a suitable technique can be evolved. The problem is to devise some form of watertight seal between the end of the intestine and a cannula inserted into it, so that fluid can be passed through the loop at constant temperature and pressure and the rate of flow and motility measured at the same time.

A satisfactory solution to this problem having been found, experiments were performed which show that when conscious dogs provided with cannulated Thiry-Vella loops of jejunum are fed a meal, changes in tone and motility occur which result in the retardation of passage of fluid such as saline or Tyrode solution. The changes have been shown to be the result of reflexes originating in the act of feeding (cephalic phase) and in the presence of food in the stomach. In addition to such influences of extrinsic origin, the contents of the loop itself have been shown to influence its motor activity with a similar result; an increase in acidity or osmotic pressure, or the presence of digestion-products or secretions, retard the transit of fluid through the loop. Evidence regarding the reflex nature of these effects is presented.

METHODS

Six dogs were provided with one or two Thiry-Vella loops of the midportion of the jejunum, the upper end of the loop being fitted with a watertight metal cannula for introduction of fluid into the loop, and the lower end exteriorized in the usual way.

The problem of evolving a suitable form of cannula proved difficult. The method recommended by Virtue & Doster-Virtue (1941), in which porous bone or stainless steel gauze is interposed between the stem of the cannula and the infolded end of the intestine, and into which connective tissue grows and forms a tight seal, was tried without success. After many attempts along various lines it became clear that the crucial point was to prevent rotation of the cannula. A form was therefore made having a widely fenestrated flange for fixation, or two bars opposite each other, through or around which connective tissue grew, preventing any movement of the cannula and resulting in a perfectly tight seal. This means of fixation has proved equally successful in other situations in the alimentary tract (gastric cannulae, pouch cannulae, etc.). The cannulae are best made by machining from a block of pure silver, the bacteriostatic action of which minimizes the discharge from the track of the cannula through the abdominal wall.

The perfusion apparatus. Most of the experiments were carried out with the apparatus shown in Fig. 1, although a simpler form of apparatus not requiring pumps was devised towards the end of the work. Fluid (0·9% NaCl or Tyrode solution) is pumped from the lower reservoir to the upper one by two Dale-Schuster or similar-type pumps and overflows down the wide central tube, the level in the upper reservoir (from which fluid passes to the loop) thus being maintained constant at a value determined by the relative positions of the

apparatus and the loop. The closed air-space in the lower reservoir communicates with a volume recorder writing on a smoked drum. The apparatus is immersed in a constant-temperature water-bath placed as close to the dog as is convenient.

As the liquid level in the lower reservoir falls, fluid is added by pipette from a reservoir in the water-bath to return the volume-recorder to its starting-point. The contractions associated with the passage of fluid through the loop are faithfully recorded on the tracing, which consists of a series of peaks, representing the refilling of the reservoir with a known volume of fluid, and a gently sloping line, on which is visible the intestinal contractions, and which indicates the rate of passage of fluid through the loop. The rate of flow may be calculated from the record and expressed graphically.

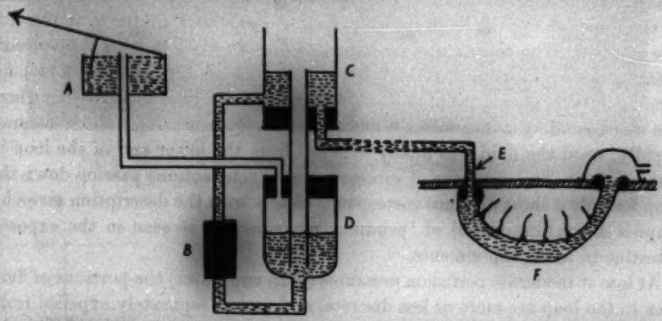

Fig. 1. Apparatus for recording the passage of fluid at constant temperature and pressure through a Thiry-Vella loop. *A*, volume recorder; *B*, pump; *C*, upper reservoir; *D*, lower reservoir; *E*, cannula; *F*, Thiry-Vella loop.

The dogs were trained to lie quietly without restraint on a warmed blanket, and the fluid issuing from the lower end of the loop led away through a wide-bore rubber tube covering the end of the loop on the body-wall but not in contact with it.

Operative technique. The only operative procedure which merits comment is that of oesophagostomy, which was carried out on one dog for the purpose of sham-feeding. Instead of using the classical method in which the oesophagus is completely divided and both ends exteriorized separately, the oesophagus was exposed and a lengthwise incision 2½–3 in. long made in it, to correspond exactly with the skin incision. The edges of the oesophagus and the skin were then united by a continuous suture of fine Nylon which was removed a few days later. Provided the incision is of ample size, there is no risk of food or fluid passing down the oesophagus into the stomach during sham-feeding. A wide-bore gastric cannula was inserted into the stomach at the same, or a previous, operation for feeding.

Meat was introduced three or more times daily through this, and fluid given through the lower end of the oesophagostomy by stomach-tube. It is essential to provide ample fluid, to offset the loss of saliva. The oesophagostomy was kept covered with a large pad of cotton-wool liberally smeared with vaseline and kept in place with a many-tailed bandage embracing the upper part of the body and the neck. Under these conditions the dogs can be kept in excellent condition indefinitely.

RESULTS

In order to obtain direct evidence of the nature of the motility associated with the passage of fluid through the loop, X-ray observations were made of this. A T-tube was inserted into the tube leading from the perfusion apparatus to the cannula, and a non-irritant radio-opaque fluid ('Pyelosil', Glaxo) run in slowly during the passage of fluid through the loop, so as to enable this to be seen on the fluorescent screen. The intestine in the vicinity of the cannula was seen to undergo a regular cycle of 'systole' and 'diastole' corresponding to the contraction waves recorded on the tracing. At systole some of the fluid constantly distending the portion of the intestine around the end of the cannula was rapidly propelled onwards by a localized contraction apparently originating at the upper end of the loop; some appeared to pass back up the tube towards the reservoir. The contraction described died away after moving a few centimetres, the ovoid mass of fluid being pushed before it coming to a halt for a moment before being swept onward once more by a second wave arising where the first ceased. Further waves followed in turn, each moving a short distance rapidly, until the fluid was finally expelled from the lower end of the loop in a short spurt. This sequence of successive rapid contractions passing down the loop for only a short distance corresponds closely with the description given by Bayliss & Starling (1899) of 'pendular movements', as seen in the exposed intestine in acute experiments.

At low or moderate perfusion pressures (5–25 cm. water) the portions of fluid seen in the loop are more or less discrete, and each is separately expelled from the lower end. As the pressure is raised, a point is reached at which fluid leaks more or less continuously through the loop, the flow from the lower end increasing with the arrival of each wave. In all the experiments reported here, the perfusion pressure was maintained at 15–20 cm. water, well below the point at which such 'leakage' occurs (35–50 cm. water). The changes in activity observed experimentally were more pronounced the lower the pressure employed for perfusion but were not otherwise influenced by it. There appears to be little if any information regarding the pressures normally developed in the small intestine during digestion, so that a 'physiological' value for the perfusion pressure could not be selected.

The contractions of the loop associated with the passage of fluid were strikingly regular in rate; the mean frequency, calculated from 324 measurements of 1 min. duration in eleven experiments, was 12·5 contractions per min., and no conditions were observed in which a significant change occurred. Douglas & Mann (1939*a*) and others have remarked on the constancy of the rhythm of the contractions observed in Biebl loops of jejunum.

The effect of a meal. Twenty-four experiments were performed, three of which were negative. In dogs fed on a diet of raw horse-flesh, milk and water, the

Fig. 2. Effect of a meal on rate of flow and motility of a Thiry-Vella loop. Tracings are consecutive. Time = 10 sec.

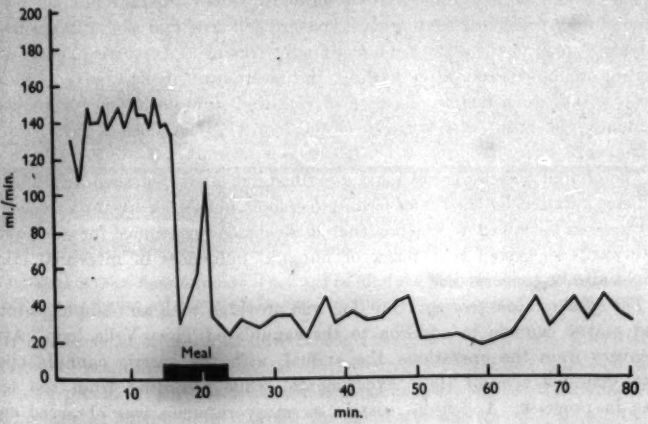

Fig. 3. The effect of a meal (250 g. cooked meat) on the flow of saline at 37° C. through a Thiry-Vella loop. Perfusion pressure 20 cm. H_2O. A transient relaxation of the intestine with increased rate of flow occurred during feeding.

loop was found to be in a 'basal' state of activity 18–24 hr. after the last meal. The flow was comparatively rapid and regular from minute to minute, with well-marked contractions of the usual rhythm visible on the record. When the animal was fed while recording was in progress, a striking change in the rate of flow and motility occurred within a minute or two of feeding, or even during this if the animal was very hungry. The flow became very much slower and the contractions increased in magnitude (Figs. 2, 3).

After a few minutes slow tonus waves appeared on the tracing and tended to become more marked during the next hour or two. These waves appeared as slow undulations in the record, indicating an emptying of fluid from the loop back into the reservoir against the perfusion pressure. X-ray observations failed to disclose any significant change in the character of the motility during the occurrence of these waves, and they would therefore appear to result from an increase in tone of the muscle, with partial emptying of the distended portion of the intestine around the cannula back into the reservoir. Occasionally in feeding and other experiments the rhythm of the contractions was interrupted by a single contraction wave of greater intensity and slower rate. These were not observed fluoroscopically on account of their infrequent occurrence; they perhaps represent peristaltic waves of classical type.

The changes in tone and motility described attained a maximum at a varying time after the meal, depending on its nature and size, and then diminished in intensity until the 'basal' state was finally regained. In three experiments, during or immediately after feeding, the retardation in flow gave place for a short time to a definite *increase* in rate and diminution in contractions, evidently the result of relaxation of the loop (Fig. 3). The cause of this is unknown.

The prompt onset of the changes described indicated that their origin lay in reflexes initiated by the act of feeding (cephalic phase), or by the presence of the meal in the stomach. The fact that these changes continued for some hours afterwards suggested that reflex or humoral influences of intestinal origin might also be concerned.

The effect of sham-feeding. One dog was provided with an oesophagostomy and gastric cannula in addition to the cannulated Thiry-Vella loop. After recovery from the operations, the animal, with the gastric cannula open, was sham-fed a meal (four experiments) while recording from the loop was in progress. A definite gastric secretory response was observed and, coincidentally with this, there occurred retardation of flow and changes in tone and motility of the Thiry-Vella loop particularly as regards the occurrence of slow tonus waves similar to those seen after feeding in the normal way (Fig. 4). The response died away within a few minutes of discontinuing sham-feeding.

The duration of the response to sham-feeding was much shorter than the response to a normal meal, showing clearly that further sources of stimulation were concerned in the latter.

The effect of gastric distension and stimulation. In two dogs provided with a gastric cannula in addition to the cannulated Thiry-Vella loop, the stomach was distended with a balloon to a pressure of 20–30 cm. water (six experiments).

Fig. 4. Effect of sham-feeding on rate of flow and motility of a Thiry-Vella loop. Note increased tonus waves during and after sham-feeding. Time signal, 10 sec. Tracings are consecutive.

A well-marked retardation in flow through the Thiry-Vella loop, with some increase in magnitude of contractions, occurred. This effect persisted for a varying period of time after the balloon was deflated and removed (Fig. 5).

When higher pressures were used, the effect was greater, and, in addition, signs of nausea (salivation, licking the lips) occurred. In view of the fact that nausea is accompanied by changes in tone and motility of Thiry-Vella loops due to vagal impulses (Gregory, 1946*a*, *b*), a few records were taken (five experiments on two dogs) of changes in tone and motility produced by minimal doses of apomorphine. These showed clearly (Fig. 6) that nausea results

in a retardation of flow in the Thiry-Vella loop and increase in contractions closely similar to the events observed after meals.

Mechanical stimulation of the gastric mucosa, by gentle stroking with a blunt-ended glass rod introduced into the stomach through the gastric cannula, produced in both dogs an increase in tone and motility which lasted for some time after stimulation ceased (Fig. 6).

Fig. 5. Effect of distension of stomach (*A*) and of mechanical stimulation of gastric mucosa (*B*) on motility and flow in a Thiry-Vella loop. Time signal, 5 sec.

That the response to feeding is not dependent solely upon gastric distension is shown by two experiments in which a dog, which displayed a very good response to feeding, was starved for 36 hr. and was then given one very small piece of cooked meat, of which it was very fond. This was given in shreds over a period of about 5 min. A striking response occurred in the loop, lasting for 3 hr. The prodecure was then repeated, with the same result. This experiment also shows clearly that the response to feeding is not an artefact due to a rise of intra-abdominal pressure resulting from filling of the stomach by the meal. Further evidence against such an interpretation of the response is afforded by the variability in the time of onset of the response in different experiments, its complete absence on three occasions when the animal was excited or unused to

the experimental conditions, and its abolition by denervation of the loop (see later).

Stimulation from the intestine after meals. Experiments to test the possibility that the response of the loop on feeding is due in part to stimulation from the

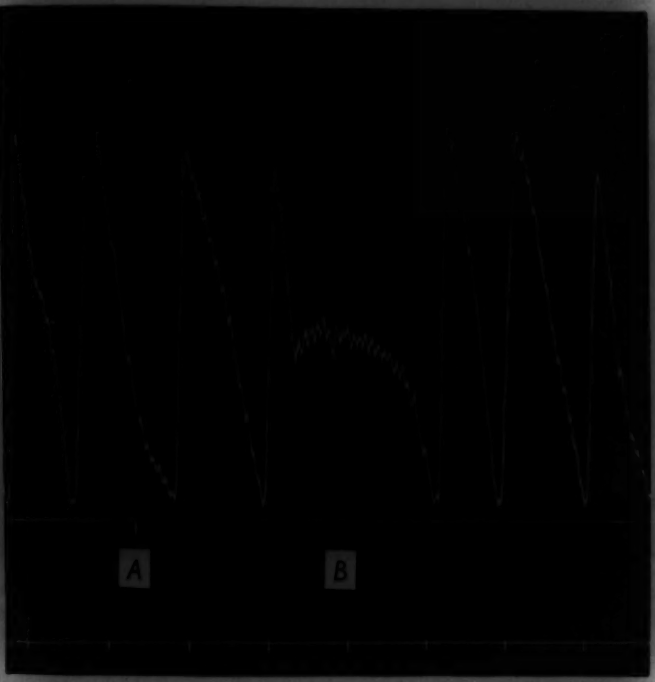

Fig. 6. Effect of nausea on flow and motility in a Thiry-Vella loop. *A*, Intramuscular injection 0·2 mg. apomorphine; *B*, signs of nausea in dog (salivation, panting). No retching. Time signal, 60 sec.

intestine, were unsuccessful. Two dogs were each provided with two Thiry-Vella loops of jejunum. Solutions of a number of substances, likely to be responsible for reflex stimulation by their presence in the rest of the intestine (acid, hypertonic saline, bile salts, protein-digests), were introduced into one loop while recording from the other was in progress. In only one out of twelve experiments was any effect observed. However, when one of the loops was distended with a balloon, or stimulated mechanically, there was no difficulty in

showing the well-known 'intestino-intestinal reflex' (Youmans, Meek & Herrin, 1938) consisting of inhibition of tone and motility in the other loop with increase in the flow through it. This response could be obtained in either direction, i.e. irrespective of which loop was stimulated.

Denervation of the intestinal loop. In order to explore the possibility that humoral agents from the stomach and intestine were concerned in the later stages of the response of the loop to a meal, the gut loop was denervated in one dog by stripping the vascular pedicle of its nerves and by painting the vessels with 1% phenol solution.

Fig. 7. Record of flow and motility after feeding a meal to a dog with a Thiry-Vella loop denervated 3 weeks previously. Perfusion pressure 15 cm. H_2O. Temperature, 37° C. Time signal, 5 sec. Distance from upper line to time signal represents 20 ml.

Unfortunately it was not possible to take recordings from this loop for nearly 2 weeks after denervation, by which time the changes in tone and motility resulting from denervation had disappeared and the behaviour of the loop differed little from normal. When a meal was fed, no significant change in tone or motility occurred within the following 6 hr. (Fig. 7).

During the ensuing 4 weeks, some degree of regeneration of the nerves appeared to occur, for, in subsequent experiments performed during this time, some signs of the response reappeared; after feeding a short-lived decrease in flow and increase in contractions often occurred, lasting for 30–60 min. followed or preceded by a transient relaxation of the intestine; a further short period of increased tone and motility also occurred about the third hour after feeding.

The full response seen before denervation had not materialized when the dog was last tested 10 weeks after denervation.

These findings must be regarded with some reserve, since the procedure was carried out on only one dog; but they appear to show clearly that the response is reflex in character and does not involve humoral agents liberated from stomach or intestine.

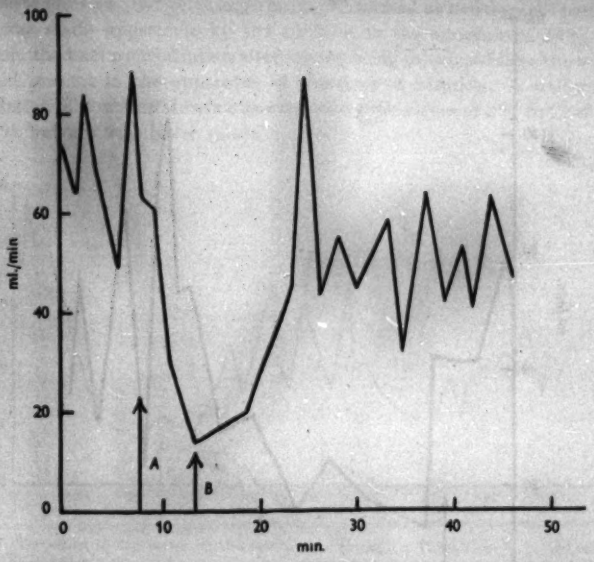

Fig. 8. The effect of acid on the flow of saline (20 cm. H_2O pressure, 37° C.) through a Thiry-Vella loop. At *A* dilute HCl was added to the reservoir; at *B* fluid entering the loop was pH 2·2.

The effect of local stimulation of the loop. The method of recording intestinal tone and motility used in these experiments is very suitable for ascertaining the effects of local influences such as acidity, osmotic pressure, and the presence of normal constituents of the intestinal contents, on intestinal tone and transportation of fluid. Experiments were therefore carried out in which the composition of the perfusing fluid was modified during recording by addition to the reservoir of various substances. The animals had in all cases been starved 18–24 hr.

Acidity. A total of eighteen experiments on four dogs were performed. After a control period of perfusion, the pH of the fluid passing through the loop was altered by adding to the lower reservoir an amount of dilute acid or alkali which had been found previously by trial to result in a pH of the desired value

when mixed with the volume of fluid in the apparatus. This took about 10 sec. As the fluid passed through the loop the reservoir was refilled with normal perfusion fluid (saline or Tyrode) so that the pH of the fluid presented to the intestine first rose or fell sharply to a peak value and then slowly returned to a pH of 7–7·5. Samples of fluid entering and leaving the loop were taken for pH measurements by the glass electrode.

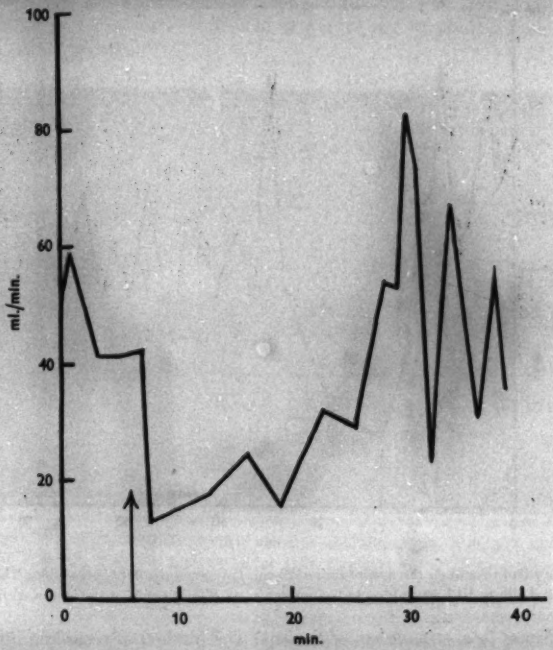

Fig. 9. The effect of hypertonicity on the flow of saline (20 cm. H_2O pressure, 37° C.) through a Thiry-Vella loop. At *A*, 10% NaCl was added to the reservoir to produce an initial concentration of 2·5% NaCl.

When the acidity was increased by adding dilute HCl to the reservoir, no consistent changes in flow were observed unless the initial change was to pH 4 or less. Then retardation in flow occurred approximately in proportion to the peak acidity (Fig. 8).

The fluid leaving the loop was always less acid than that entering, and was usually pH. 5·5–6, sometimes higher, indicating that passage of fluid through the loop was dependent upon its neutralization, presumably by the secretion of

intestinal juice. This is supported by the observation that, for a given initial pH, the effect on the flow was more pronounced and of longer duration when the perfusion fluid was buffered (Tyrode) than when it was not (saline).

When the alkalinity was increased by the addition of dilute NaOH, retardation of flow was observed with peak pH values of 9 or more, but not consistently when the fluid was less alkaline than this.

Osmotic pressure. In twelve experiments performed on two dogs the perfusing fluid was made hypertonic by the addition to the reservoir of 10% NaCl solution, the final concentration after mixing being calculated from the volume of fluid present in the apparatus at the time of addition. A well-marked retardation of flow was always observed with peak values of 2% NaCl or more (Fig. 9), but not with lower values.

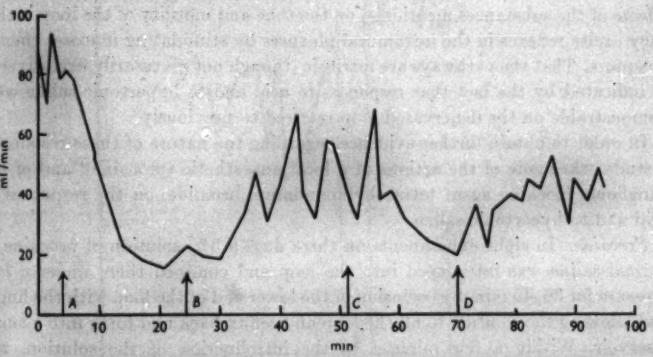

Fig. 10. The effect of tap water on the rate of flow through a Thiry-Vella loop. (20 cm. H_2O pressure, 37° C). At *A* and *C* the normal perfusion fluid (Tyrode) was changed for tap water. At *B* and *D* the reverse change was made.

Water. (Seven experiments on three dogs). Changing the perfusion fluid to tap water by emptying the apparatus as completely as possible and refilling with water produced a marked retardation in flow (Fig. 10). No precise information was obtainable regarding the threshold of hypotonicity for stimulation, but the impression was gained that the intestine is somewhat less sensitive to a fall in osmotic pressure than to an increase.

Other substances. Fourteen experiments were performed on three dogs in the way described above. Solutions in saline of bile salts, of soap, of a pancreatic digest of protein, or of neutralized duodenal contents taken from a dog provided with a duodenal fistula, were used. These experiments are not reported in detail, since the results are essentially similar to those obtained with acid and

with hypertonic solutions, etc. All these substances, when added to the perfusion fluid, could be shown to produce retardation of flow through the loop. The effects were not due to changes in acidity or in osmotic pressure, since these effects occurred when the changes in pH or osmotic pressure were well below the threshold values previously established.

It seems likely that, if minimal perfusion had been used in these experiments, smaller deviations from the normal, in respect to the factors studied, could have been shown to produce significant effects upon the rate of flow. No attempts were made to determine the minimal effective changes in composition; but the results obtained demonstrate the existence in the intestine of a mechanism for retarding the passage of fluid which deviates from neutrality and isotonicity, or contains digestion products and secretions.

The mechanism of the local responses. The most likely explanation for the local effects of the substances mentioned on the tone and motility of the loop is that they excite reflexes in the intramural plexuses by stimulating mucosal chemoreceptors. That the pathways are intrinsic (though not necessarily exclusively), is indicated by the fact that responses to acid and to hypertonic saline were demonstrable on the denervated loop referred to previously.

In order to obtain further evidence regarding the nature of these responses, a study was made of the actions of a local anaesthetic (procaine), and of the ganglionic blocking agent tetraethylammonium bromide, on the responses to acid and to hypertonic saline.

Procaine. In eight experiments on three dogs a 5% solution of procaine in normal saline was introduced into the loop and confined there under a low pressure for 30–40 min. by occlusion of the lower end of the loop with the finger and allowing the solution to fill the loop and reflux back and forth into a small reservoir. Within a few minutes of the introduction of the solution, the rhythmical contractions largely ceased and the loop became quiescent and relaxed. Raising the reservoir provoked one or two contractions but no more. After this period, perfusion of the loop with normal saline was carried out in the usual way. The loop was found to be relaxed and the rate of flow very rapid, although weak contractions of the usual frequency could be detected on the record. The addition of acid or of hypertonic saline in amounts which produced well-marked changes in flow before procainization had now no effect on the rate of flow or motility (Fig. 11).

After a varying interval of time, usually about 30 min. from the start of perfusion with normal saline, short periods of normal tone and motility occurred, interspersed with relaxation and rapid flow. In this way the normal behaviour of the intestine was gradually resumed. A few hours afterwards the same amounts of acid and of hypertonic saline again exerted their characteristic effects. No systemic effects referable to absorption of procaine from the loop were observed.

Tetraethylammonium bromide (TEAB). In nine experiments on three dogs, the rapid intravenous injection of this drug in amounts of 10–15 mg./Kg. body weight in a single dose resulted in prompt relaxation of the loop with a sudden and sustained increase in flow and almost complete disappearance of contractions. After a variable period of time, usually about 10–15 min. with the dose mentioned, short periods of normal flow and motility were observed interspersed with periods of relaxation, and the normal state of the loop was

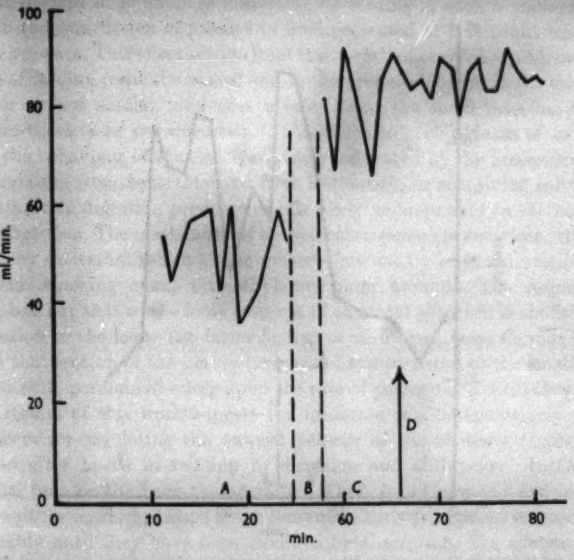

Fig. 11. The effect of procaine on the response of a Thiry-Vella loop to acid. Perfusion pressure, 15 cm. H_2O; temperature 38° C. throughout. *A*, Control; *B*, 40 min. exposure of mucosa to 5% procaine in saline; *C*, rapid flow after procaine; *D*, addition of acid to reservoir.

subsequently rapidly regained. During the period of apparent effective action of the drug, i.e. while the intestine was fully relaxed, the addition to the perfusing fluid of acid or hypertonic saline still resulted in some retardation of flow, indicating that the action of these agents had not been blocked by the drug, although in some cases the response appeared to have been diminished (Fig. 12).

The response of the intestine to nausea, produced by the injection of apomorphine, was likewise not blocked by TEAB (three experiments on two dogs).

The results of the procaine experiments support the hypothesis that the local intestinal responses are the result of reflexes which originate in the stimulation of mucosal receptors. TEAB, however, failed to prevent these responses

when administered in doses which are adequate to block ganglionic transmission in other sites. This might be regarded as indicating that the pathways involved in the response are not a true reflex arc but are possibly of the nature of an 'axon reflex'. Hill (1927), in her study of the intrinsic plexuses of the gut, was unable to find any histological evidence of reflex arcs and was inclined to agree with the suggestion of Muller (1892) that the peristaltic response of the intestine was of the nature of an 'axon reflex'.

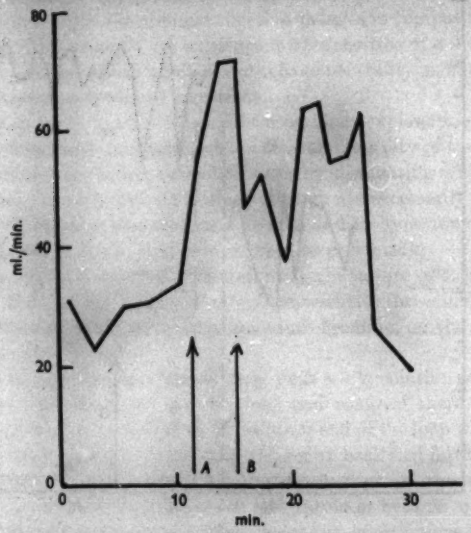

Fig. 12. Effect of tetraethylammonium bromide (10 mg/kg. intravenously) on response of a Thiry-Vella loop to acid. Perfusion pressure 15 cm. H_2O, temperature 37° C. throughout. *A*, injection of TEAB; *B*, addition of acid to reservoir.

However, TEAB did not block the intestinal response to nausea, which has been shown (Gregory, 1946*b*) to be due to vagal excitation; and Oppenheimer (1946) and Whitrock & Roe (1948) (quoted by Moe & Shideman, 1949) found that TEAB does not block the response of the duodenum to vagal stimulation. These findings indicate that the ganglion cells which constitute the motor pathway to the intestinal muscle for impulses of either intrinsic or extrinsic origin are not rendered inexcitable by the drug. The results of the TEAB experiments do not therefore throw any light on the nature of the nervous pathways involved in the local responses of the intestine acid and to hypertonic solutions. The interpretation of the results is further complicated by the fact that TEAB will

block reversibly the peristaltic reflex excited by distension in the Trendelenburg preparation of the isolated guinea-pig ileum (Gregory, 1949); but, on the other hand, the type of motility involved in the intestinal responses studied in the present experiments is almost certainly different in nature from the peristaltic reflex excited by distension.

DISCUSSION

In the experiments reported it has been shown that the tone and motility of segregated loops of jejunum is influenced by feeding in such a manner as to result in the retardation of passage of fluid presented at a constant moderate head of pressure. This effect results from the operation of reflexes originating in the act of feeding (cephalic phase) and in the presence of food in the stomach. Whether or not similar influences operate from the small intestine during digestion there is as yet uncertain. In addition to such reflexes of extrinsic origin, the behaviour of the loop is similarly influenced by the presence in it of fluid deviating from neutrality and from isotonicity, or containing substances such as protein digestion products or bile likely to be present in the intestine during digestion. The mechanism of these local responses is uncertain; they are blocked by anaesthetization of the mucosa but not by administration of the ganglionic blocking agent tetraethylammonium bromide. The response to a meal, but not that to the local presence of chemical agencies, is abolished by denervation of the loop. The latter finding is of interest, since Cannon (1906) showed that section of the nerves (vagus and sympathetic) to the small intestine had little permanent effect upon the rate of passage of a meal through it.

The results of this work suggest the existence of a comparatively simple mechanism for regulating the onward passage of the contents of the small intestine after meals in relation to digestion and absorption. Increase in intestinal tone results from the operation of reflexes of extrinsic and intrinsic origin, and the onward passage of the contents is thereby impeded or prevented, presumably until they have been modified by absorption. The efferent pathways of the extrinsic reflexes have not been defined experimentally, but in view of the general nature of the response (increase in tone and motility), and the similarity of the response during nausea, it seems likely that the vagi are concerned.

In the present experimental conditions, the response described has been shown in operation against a constant head of pressure tending to force fluid through the loop, as though from a region of the intestine orad to it; and in seeking to relate the findings to conditions in the intact intestine, the question arises as to the physiological counterpart of this filling pressure. In other words, what are the forces tending to propel the contents of the intestine onwards and how are they controlled and correlated with the restraining mechanism disclosed in the present work? It is difficult to escape the conclusion, admittedly speculative, that one such factor is to be found in the propulsive motility of the

stomach, excited as it is after a meal by increased vagal tone, and restrained by the operation of the 'enterogastric reflex' (Thomas & Mogan, 1931) and by the liberation of enterogastrone from the upper part of the small intestine; but so far as the intestine itself is concerned, valuable information would be obtained by investigation of the effect of the conditions studied in the present work on the *propulsion* of fluid through isolated loops. This can be done by raising the pressure at the caudal end of the loop to a value higher than the pressure of the inflow, so that the loop is subjected to distension throughout, and fluid cannot pass unless it is propelled against the pressure gradient existing between the two ends of the loop.

Some preliminary experiments have already been made along these lines.

SUMMARY

1. A method is described for recording the rate of flow of fluid at constant temperature and pressure through Thiry-Vella loops of jejunum in conscious dogs.

2. Feeding a meal causes a sustained increase in tone and in motility, resulting in retardation of the flow of fluid through the loop.

3. These changes are of reflex origin and have been shown to be due to (*a*) the act of feeding (cephalic phase), and (*b*) the presence of food in the stomach. Whether stimulation from the intestine is also concerned is uncertain.

4. Denervation of the vascular pedicle of the loop abolishes the response to feeding (one dog).

5. Nausea produced by the injection of minimal amounts of apomorphine causes changes in intestinal tone and motility similar to those observed after feeding.

6. The presence in the loop of fluid which deviates appreciably from neutrality and isotonicity, or which contains digestion-products or secretions, likewise results in changes in tone and in motility, and in the flow of fluid through the loop, similar to those caused by feeding.

7. The responses to the presence in the loop of acid or of hypertonic solutions are still demonstrable after denervation.

8. Anaesthetization of the mucosa of the loop by exposure to a 5% procaine solution abolishes temporarily the local responses to acid and to hypertonic solutions.

9. The intravenous injection of tetraethylammonium bromide does not prevent the local responses of the loop to acid and to hypertonic solutions, nor does it prevent the changes in tone and motility associated with nausea.

10. The results suggest that a mechanism, partly extrinsic and partly intrinsic in nature, may exist in the intact intestine by which the onward passage of its contents after a meal is restrained. Moreover, the degree of this restraint seems to depend partly on the composition of the intestinal contents and partly on the changes produced by digestion and absorption.

REFERENCES

Alvarez, W. C. (1940). *An introduction to Gastro-Enterology*, 3rd ed. London: Heinemann.
Barcroft, J. & Robinson, C. S. (1929). *J. Physiol.* **67**, 211.
Bayliss, W. M. & Starling, E. H. (1899). *J. Physiol.* **24**, 99.
Cannon, W. B. (1904). *Amer. J. Physiol.* **12**, 387.
Cannon, W. B. (1906). *Amer. J. Physiol.* **17**, 429.
Castleton, K. B. (1934). *Amer. J. Physiol.* **107**, 641.
Douglas, D. M. & Mann, F. C. (1939*a*). *Amer. J. Dig. Dis. Nutrit.* **6**, 318.
Douglas, D. M. & Mann, F. C. (1939*b*). *Amer. J. Dig. Dis. Nutrit.* **6**, 434.
Douglas, D. M. & Mann, F. C. (1940). *Amer. J. Dig. Dis. Nutrit.* **7**, 53.
Gregory, R. A. (1946*a*). *J. Physiol.* **105**, 58.
Gregory, R. A. (1946*b*). *J. Physiol.* **106**, 95.
Gregory, R. A. (1949). Unpublished experiments.
Hill, C. J. (1927). *Philos. Trans.* B, **215**, 355.
Moe, G. K. & Shideman, F. E. (1949). *Ann. Rev. Physiol.* **11**, 567.
Muller, E. (1892). *Arch. mikr. Anat.* **40**, 216.
Thomas, J. E. & Mogan, C. J. (1931). *Proc. Soc. exp. Biol., N.Y.*, **28**, 968.
Virtue, M. E. & Doster-Virtue, R. W. (1941). *Proc. Soc. exp. Biol., N.Y.*, **48**. 326.
Youmans, W. B., Meek, W. J. & Herring, R. C. (1938). *Amer. J. Physiol.* **124**, 470.

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THE INORGANIC COMPONENTS OF GASTRIC SECRETION

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Many attempts have been made to account quantitatively for the observed variations in composition of gastric juice, the most recent being those made by Hollander (1938), Gray, Bucher & Harman (1941) and Gray & Bucher (1941). Almost all the work in this field has been done on dogs, and has tacitly assumed (*a*) that the acid parietal cell secretion is of constant composition, so far as HCl and neutral chloride are concerned, and (*b*) that the remainder of the secretions, referred to as non-parietal, are not only constant in composition with respect to bicarbonate and chloride, but are also secreted at constant rates.

In connexion with other work we have been faced with the necessity to find some specification of gastric secretory activity in man in terms of acid, neutral chloride, and volume secreted. We have taken the extensive data of Ihre (1938) on gastric secretion in selected young men in response to histamine or insulin administration as a basis for testing possible theoretical relations between amounts of acid, neutral chloride, and total volume of juice secreted.

Basic assumptions. In this work we have started with less restrictive assumptions than those referred to above. The primary assumptions here are: (1) that the parietal secretion contains HCl and neutral chloride in constant concentrations; (2) that the mixed non-parietal secretions contain neutral chloride and bicarbonate in constant concentrations.

No assumptions are made about the constancy or otherwise of the rate of secretion of either parietal or non-parietal secretions. There is, however, a further tacit assumption, common to this work and to earlier work on dogs, that no changes in composition occur in the mixed gastric juice whilst it remains in the stomach. Ihre's data are based on samples continuously withdrawn from the stomach, which reduces to a minimum the opportunity for the exchange of ions across the gastric mucosa.

Theoretical relations between composition and volume

Denoting the concentrations of acid, neutral chloride, and bicarbonate in the component secretions and the amounts of acid and neutral chloride in the mixed juice as follows:

Parietal secretion	Non-parietal secretions	Mixed juice
h = conc. of acid	b = conc. of bicarbonate	A = amount of acid m.equiv.
p = conc. of neutral chloride	c = conc. of neutral chloride	N = amount of neutral chloride m.equiv.

and putting

x = volume of parietal secretion in period of observation,

y = volume of non-parietal secretion in period of observation,

V = volume of mixed juice secretion in period of observation,

we have $$V = x + y, \quad \text{(i)}$$

$$A = hx - by, \quad \text{(ii)}$$

and $$N = px + cy + by, \quad \text{(iii)}$$

provided the juice is acid.

These three equations constitute the fundamental relations, and the further development is largely a matter of algebraic modification of them.

Solving equations (ii) and (iii) for x and y gives

$$x = \frac{bA + cA + bN}{bh + ch + bp}, \quad y = \frac{hN - pA}{bh + ch + bp},$$

whence $$V(=x+y) = \frac{h + c - p}{bh + ch + bp} A + \frac{b + h}{bh + ch + bp} N. \quad \text{(iv)}$$

Since the coefficients of A and N in (iv) are functions of quantities assumed to be constant, this equation can be written more compactly as

$$V = K_1 A + K_2 N, \quad \text{(v)}$$

which expresses the general form of the relation between volume, total acid, and total neutral chloride content of gastric juice which follows from the assumptions made.

Tests of fit of the fundamental theory to observation

Two tests of the fit of equation (v) to Ihre's published data are possible.

(1) The most general first-order relation between the three observable variables V, A and N is of the form

$$V = k_1 A + k_2 N + k_3, \quad \text{(vi)}$$

but the particular first order relation required by the theory requires that k_3 should be zero. The best fit of Ihre's two series of observations on normal subjects (twenty-four observations on secretion in response to histamine, twenty-four observations on secretion in response to insulin, both on the same set of subjects, each series giving totals for three consecutive 20 min. periods) to the most general type of relation of the form of equation (vi) was computed, and the magnitude and error of estimation of k_3 was obtained for each series. The values obtained were: histamine secretion, $k_3 = 5{\cdot}86 \pm 3{\cdot}13$ ml./hr; insulin secretion, $k_3 = 4{\cdot}34 \pm 2{\cdot}30$ ml./hr.

Neither of these estimates is significantly different from zero. Their significance is best judged by noting that they represent those portions (in ml./hr.) of the total volume secreted per hour which cannot be accounted for on the assumption that the mixed juice is a mixture of two components of constant

composition. Since the mean rates of secretion in the two series are 102 and 132 ml./hr. respectively, it is clear that the k_3 terms are not only not statistically significantly different from zero but also quite trivial in relative magnitude.

(2) The second test is to plot for all observations the volumes estimated by the best fit relation of the theoretical form ($V = k_1A + k_2N$) against the observed volume. Should the theoretical relation be only a rough approximation to the true relation, such a plot should disclose this: if, on the other hand, the calculated volume corresponds equally closely to the observed volume over the whole range of observed volumes, the observations can be taken to be systematically consistent with the theory.

As a prelude to this test the best-fit relations of the form $V = k_1A + k_2N$ were computed for both series. The values of the constants for the two series were found not to differ significantly (Table 1), despite the difference in the manner

TABLE 1. Values of the constants obtained by fitting the equation $V = k_1A + k_2N$ to Ihre's (1938) observations on normal male subjects

	k_1	k_2
Histamine juice	5·63±0·24	8·16±0·74
Insulin juice	5·98±0·12	6·52±0·43
Differences	0·35±0·29	1·64±0·83

(Twenty-four observations in each series.)

of exciting secretion. The data for the two series were pooled, in view of the similarity of the equations, and Fig. 1 shows the correspondence of volumes calculated from this pooled relation and the observed volumes. Both series show a uniform correspondence of estimated with observed volumes over the whole range of observed volumes available (50–204 ml./hr.).

On the basis of these two tests it is reasonable to conclude that Ihre's observations are in conformity with the theory set out here. The theory is not proved, but the available observations can be accounted for in terms of it.

Composition of components of gastric secretion

'*Extrapolation methods.*' Much of the work on dogs referred to in the introduction to this paper was directed to attempts to determine the composition of the parietal secretion. In particular, earlier workers have found that, by plotting free-acid concentration against the reciprocal of the volume, a straight line is obtained. The acid concentration obtained by extrapolating this line to $1/V = 0$ has been taken as the free-acid concentration in the parietal component.

Such a relationship can be derived from the theory set out here on the basis of an additional assumption, namely, that the rate of secretion of the non-parietal component is constant.

Put the volume of non-parietal component secreted in the period of observation at a constant value y, and substitute $V-y$ for x in equations (ii) and (iii), obtaining:

$$A = hV - (b+h)y, \tag{vii}$$

$$N = pV + (c+b-p)y, \tag{viii}$$

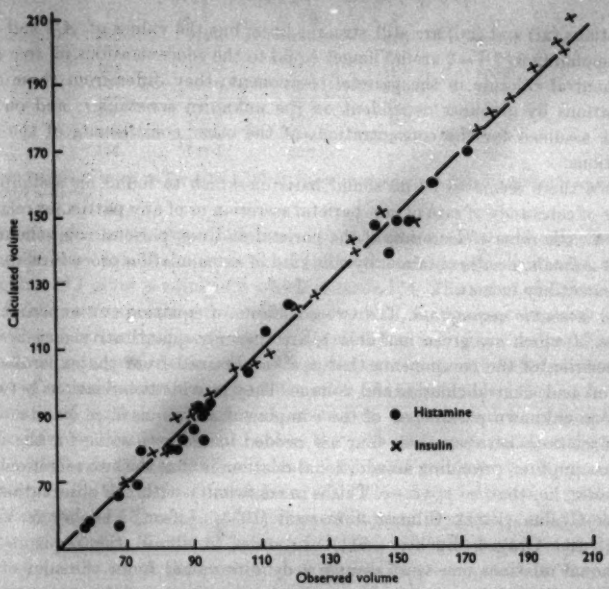

Fig. 1. The correspondence of calculated to observed volume. The abscissa is the volume observed, and the ordinate the volume calculated from the equation $V = 5{\cdot}79\,A + 7{\cdot}41\,N$, the pooled relation for all observations on normal subjects.

or, on dividing both these equations through by V,

$$A/V = h - (b+h)y.1/V, \tag{ix}$$

and

$$N/V = p + (c+b-p)y.1/V. \tag{x}$$

These equations give linear relations such that the values of A/V and N/V corresponding to $1/V = 0$ are respectively the concentrations of acid and neutral chloride in the parietal component.

But if it should be the case that the non-parietal component is secreted at a rate which shows any systematic relation to rate of parietal secretion, this convenient relation would no longer hold. Suppose, for instance, that the rate

of non-parietal secretion is related to the rate of gastric juice secretion in a manner approximating to $y=rV+s$, where r may be either positive or negative, and both r and s are constants. Then equations (ix) and (x) become:

$$A/V=(h-rb-rh)-(b+h)s.1/V, \tag{xi}$$

and

$$N/V=(p+rc+rb-rp)+(c+b-p)s.1/V. \tag{xii}$$

Equations (xi) and (xii) are still straight lines, but the values of A/V and N/V corresponding to $1/V=0$ are no longer equal to the concentrations of free acid and neutral chloride in the parietal component: they differ from these concentrations by amounts dependent on the unknown constant r, and on the values assumed for the concentrations of the other constituents of the two secretions.

Since there seems to be no sound basis on which to found an assumption either of constancy of rate of non-parietal secretion or of any particular relation between the rates of secretion of the parietal and non-parietal components in intact animals, results obtained by this kind of extrapolation procedure are not of great utility in man.

The isosmotic assumption. The two constants of equation (v), experimental values of which are given in Table 1, are the only quantitative measures of composition of the components that can be obtained from the experimental data on acid, neutral chloride and volume. They provide two relations between the four unknown parameters of the component secretions h, p, b and c. Two other relations between these four are needed in order to solve for the four. One assumption, providing an additional relation, is that the two secretions are isosmotic, i.e. that $h+p=b+c$. This is in conformity with the observations of Ivy & Oyama (1921), Gilman & Cowgill (1933), Lifson, Visscher & Varco (1943) and Gray & Bucher (1941). In order to obtain the requisite two additional relations one must assume a definite value, I, for the sum of the concentrations of the constituents, so that

$$h+p=I, \tag{xiii}$$

$$b+c=I. \tag{xiv}$$

Combining these with

$$k_1=\frac{b+c-p}{bh+ch+bp}, \tag{xv}$$

and

$$k_2=\frac{b+h}{bh+ch+bp} \tag{xvi}$$

(which are the equations of the experimentally measurable constants of equation (v) with their theoretical forms set out in equation (iv)), it is possible to write expressions for h, p, b, and c in terms of the k's and of an assumed value of I. These expressions are:

$$\left.\begin{aligned} h&=(k_2I-1)/(k_2-k_1), & p&=(1-k_1I)/(k_2-k_1),\\ b&=(k_2I-1)/k_1, & c&=(1+k_1I-k_2I)/k_1. \end{aligned}\right\} \tag{xvii}$$

Using a series of arbitrarily selected values of I, and the experimentally determined pooled values of k_1 and k_2, the values obtained for h, p, b and c are those listed in Table 2.

TABLE 2. Estimates of concentrations of constituents of components of human gastric juice corresponding to different values of I. All concentrations in m.equiv./l.

I	h	b	p	c
160	114·6	32·2	45·4	127·8
162	123·7	34·7	38·3	127·3
164	132·9	37·3	31·1	126·7
166	142·0	39·9	24·0	126·1
168	151·1	42·4	16·9	125·6
170	160·2	45·0	9·8	125·0
172	169·4	47·5	2·6	124·5
174	178·5	50·1	—	123·9

(These values are based on $k_1 = 5{\cdot}79$, $k_2 = 7{\cdot}41$, the estimates derived from the pooled observations on normal subjects.)

Quantitative consequences of the isosmotic hypothesis. It would appear that I must be less than 174 m.equiv./l., if the isosmotic hypothesis is valid, since this value of I gives a value of h which exceeds 174. The value of I for which $h = I$ is $1/k_1$, corresponding to $I = 172{\cdot}7$ m.equiv./l. for the present data.

Dr Ihre has kindly placed at our disposal the individual values for the separate 20 min. periods on which his published figures are based, and these permit of somewhat more precise estimation of the probable value of I. For instance, the highest concentration of acid to be expected in a sample of gastric juice will be less than h, unless the juice is pure parietal component, but 18 of Ihre's 238 individual values have acid concentrations exceeding 140 m.equiv./l., the highest value being 151. This suggests that h is probably rather in excess of 151 m.equiv./l., corresponding to I in excess of 168 (Table 2). On the other hand, the total Cl^- of mixed juice will be expected to reach the value of I only when the juice is pure parietal secretion, and in only four instances out of 238 was the observed total Cl^- in excess of 170 m.equiv./l. Combining these observations, I is probably greater than 170, though probably not much greater, and must be less than 172·7 m.equiv./l.

There is one other possible way of fixing the probable value of I. In Ihre's data the minimum concentration of neutral chloride recorded is 16 m.equiv./l. There are thus no observations which demand the assumption that the parietal component contains no metallic cations. From the work of Gray & Bucher (1941) it appears that the parietal component, at least in dogs, contains 7 m.equiv./l of potassium. They arrived at this value from the results of an analysis of gastric juice collected from the stomachs of vagotomized dogs after the injection of histamine. In spite of variations in the rate of secretion and of the proportion of parietal component to non-parietal component the concentration of potassium remained constant at 7 m.equiv./l. They thus came to the conclusion that the concentration of potassium was the same in both compo-

nents. In man it is difficult to collect gastric juice without contamination with saliva, which contains at least 16 m.equiv./l. of potassium. It is therefore not surprising to find that the recorded concentrations of potassium in human gastric contents vary. But the lowest concentration recorded might be expected to give some indication of the concentration in the parietal component if Gray & Bucher's work in dogs is applicable to man. The lowest recorded concentration that has come to light is 10 m.equiv./l. reported by Katsch, Baltzer & Brink (1934). This estimate is confirmed by the observations of Kozawa, Fukushima, Umeno, Kurihara, Takata & Horiuchi (1933). These authors investigated gastric content, not continuously aspirated gastric juice. If those of their samples in which the Cl^- concentration is less than 125 m.equiv./l. are excluded, since they were probably contaminated by extra-gastric fluids, the mean K content of the remaining samples is $12 \pm 0{\cdot}6$ m.equiv./l. If it be assumed that the value of p, the neutral chloride concentration in parietal secretion, can be equated with the minimal K concentration in gastric juice, then both these series of observations correspond to values of I in the region of 170 m.equiv./l. (See Table 2 for the relation between p and I.) It would seem then that if the isosmotic hypothesis is valid for man, the estimates for Ihre's group of subjects give approximately:

Isosmotic conc. of Cl^-	170	m.equiv./l.
Parietal H^+ concentration	160	,,
Parietal neutral Cl^- conc.	10	,,
Non-parietal neutral Cl^- conc.	125	,,
Non-parietal HCO_3^- conc.	45	,,

It is particularly suggestive that three different criteria, the maximum observed acid concentration, the maximum observed total Cl^- concentration and the minimum observed potassium concentration should all indicate approximately the same value of I. However, as will be shown later, this does not mean that the estimates of h, p, b and c are correspondingly closely determined.

Application to other groups of subjects

The group of Ihre's subjects providing the data used to test the theoretical relations developed here was a selected group of young male students. The quantitative deductions above concerning the composition of the components of human gastric juice may therefore not be of general application.

Some check on this can be made by using a transformation of the fundamental equation developed in this paper. If the concise form, equation (v), is divided through by V, we have

$$1 = k_1 A/V + k_2 N/V,$$

or

$$A/V = 1/k_1 - (k_2/k_1)(N/V), \qquad \text{(xviii)}$$

which is a linear relation between acid concentration and neutral chloride concentration, the parameters of which are functions of the composition of the component secretions. Both the slope and intercept of this line are functions of all four of h, p, b and c, so that both the slope and intercept should be altered by any appreciable variation in the composition of either parietal or non-parietal secretion. Thus a plot of A/V, i.e. concentration of acid, against N/V, i.e. concentration of neutral chloride, either for a group of subjects or for a group of observations on a single subject should provide a visual test of fit or divergence from the relation found here for Ihre's subjects.

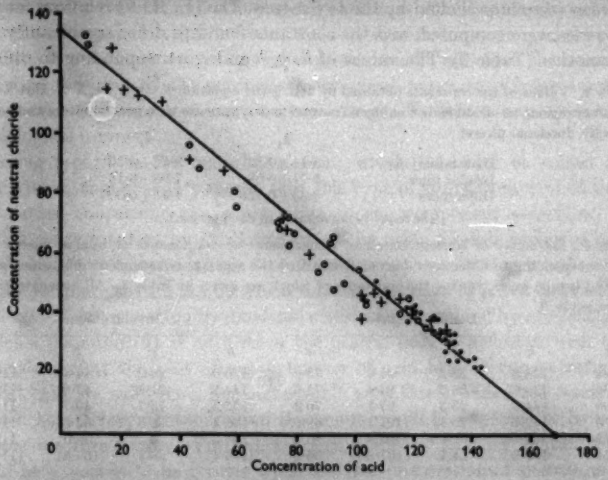

Fig. 2. The relations between acid concentration and neutral chloride concentration. The line represents the pooled relation for normal subjects, and the points show the agreement with this relation of individual observations from normal subjects, patients with gastric ulcer and patients with duodenal ulcer. •, normal subjects; ○, patients with gastric ulcers; +, patients with duodenal ulcers.

In fig. 2 the concentration of neutral chloride has been plotted against the concentration of acid in samples of gastric juice obtained by Dr Ihre from twenty-four normal subjects, and forty patients with peptic ulcers, after stimulation with insulin. It may be seen that all the points fit well on to the line which was computed for normal subjects. This fit is all the more remarkable since the composition of the pathological juices differs widely from that of the normal juices, and since there is in the pathological group no selection of subjects by age or by copiousness of secretion. Thus the quantitative relationships set up for a selected group of normal young subjects are also true of patients with peptic ulcers with a mean age of 40.

This point has been investigated further and it has been shown that the best fit planes through the origin describing the (V, A, N) relations of Ihre's series of normal subjects, gastric ulcer patients and duodenal ulcer patients are statistically indistinguishable.

Thus the relationships described earlier would seem to be generally valid, not merely applicable to a selected group of young males. We may therefore suppose that all the observations from normal and peptic ulcer patients may be reclassified into those on histamine juice and those on insulin juice, and the two series of seventy observations may be used to test more rigorously for differences between secretions elicited by the two means. The (V, A, N) relations for these two series were computed, and the constants found to differ significantly from one another (Table 3). The values of h, p, b and c, corresponding to different

TABLE 3. Values of the constants obtained by fitting the equation $V = k_1A + k_2N$ to Ihre's (1938) observations on all subjects combined (normal males, patients with gastric ulcers and patients with duodenal ulcers)

	k_1	k_2
Histamine juice	$5{\cdot}44 \pm 0{\cdot}12$	$9{\cdot}12 \pm 0{\cdot}26$
Insulin juice	$5{\cdot}77 \pm 0{\cdot}08$	$7{\cdot}66 \pm 0{\cdot}16$
Differences	$0{\cdot}33 \pm 0{\cdot}128$	$1{\cdot}46 \pm 0{\cdot}307$

(Seventy observations in each series.)

TABLE 4. Estimates of concentrations of constituents of components of human gastric juice, corresponding to different values of I, based on the separate equations for histamine-excited and insulin-excited juice, the constants of which are given in Table 3. All concentrations in m.equiv./l.

	Histamine juices				Insulin juices			
I	h	p	b	c	h	p	b	c
164	134·8	29·2	91·0	73·0	136·0	28·0	44·4	119·6
166	139·8	26·2	94·4	71·6	144·2	21·8	47·0	119·0
168	144·7	23·3	97·7	70·3	152·3	15·7	49·7	118·3
170	149·7	20·3	101·1	68·9	160·4	9·6	52·3	117·7
172	154·6	17·4	104·4	67·6	168·5	3·5	55·0	117·0
174	159·6	14·4	107·8	66·2	(176·7)	(—)	(57·8)	(116·4)

values of I were, therefore, computed for these new equations and are given in Table 4. It will be seen that the estimates of composition of the parietal secretion (h and p) do not differ greatly for the two kinds of juice, but that the estimates of non-parietal composition (b and c) differ considerably. It is difficult to evaluate the errors of estimation of these quantities, but it seems that the standard errors of the estimates of h and p must be in the neighbourhood of ± 10 m.equiv./l., whilst the standard errors of the estimates of b and c are probably somewhat in excess of ± 5 m.equiv./l. Thus, the parietal component composition is probably not significantly different in the two instances, whereas the composition of the non-parietal secretions probably does depend significantly on the nature of the agent exciting secretion.

The second point to be tested with the larger number of observations is the validity of the equation $V = k_1A + k_2N$. Best-fit planes of the form $V = k_1A + k_2N + k_3$ were fitted to the pooled histamine observations and to the

pooled insulin observations. The values of k_3 obtained were $-1{\cdot}70 \pm 1{\cdot}83$ and $+0{\cdot}07 \pm 2{\cdot}26$ respectively for the two series of seventy observations. Thus there is no conflict with the theoretical requirement of $k_3 = 0$.

Utility of the quantitative relations described

Although there seems to be ground for supposing that the theoretical relations set out in this paper describe adequately the relations between volume, total acidity, the neutral chloride content of gastric secretion, it appears that these relations will not, of themselves, yield estimates of the composition of parietal and non-parietal secretions. The further assumptions of (*a*) the isosmotic relation between the component secretions, and (*b*) of an arbitrary isosmotic concentration must first be made, and then the estimates of the composition of the component secretions prove to involve errors of estimation which are too great to leave to the estimates any appreciable physiological interest.

Having regard to the remarkable pains which Ihre took to collect pure gastric juice, this probably means that this type of study cannot yield useful information concerning the composition of the component secretions. He avoided contamination by swallowed saliva, by using a saliva-aspirator in the mouth, and by not beginning his experiments until the collecting tube had been in place for more than 1 hr., by which time the secretion of saliva had fallen to low levels. Contamination by duodenal contents was avoided by aspirating the duodenal contents. It is only when the gastric juice is collected with these precautions that the plot of concentration of acid against concentration of neutral chloride will give a line comparable with Ihre's. When the results are vitiated by salivary contamination the points would be expected to lie below the line for Ihre's data. Further, the significance of salivary contamination would be expected to be greater when the rate of secretion of gastric juice is low, which is tantamount to saying when the concentration of acid is low. In connexion with other work, histamine tests were performed by one of us (J.N.H.) on twenty-one normal subjects. Gastric juice was aspirated continuously in these tests, and there was effective salivary aspiration. However, the tests were begun shortly after passing the stomach tube and not after 1 hr. as in Ihre's experiments. No attempt at continuous duodenal aspiration was made. Fig. 3 shows the results of these tests plotted in the same way as in Fig. 2. It may be seen that the expected trend of the results away from the line for Ihre's normal subjects is shown in spite of continuous salivary aspiration from the mouths of these trained subjects.

The relation described has proved useful, however, in gastric emptying studies to estimate the total volume of secretion added to the gastric contents, and it may be used with a modicum of confidence to estimate the probable volume of a component secretion, given a reasonable reliable estimate of the

isosmotic concentration, I. Referring back to equations (ii) and (iii), the expression for y, the volume of non-parietal secretion, may be combined with the equation (xvii), relating h, p, b and c to k_1, k_2 and I to yield the following expression

$$y = k_1\{(k_1 I - 1)/(k_2 I - 1)\}A + N.$$

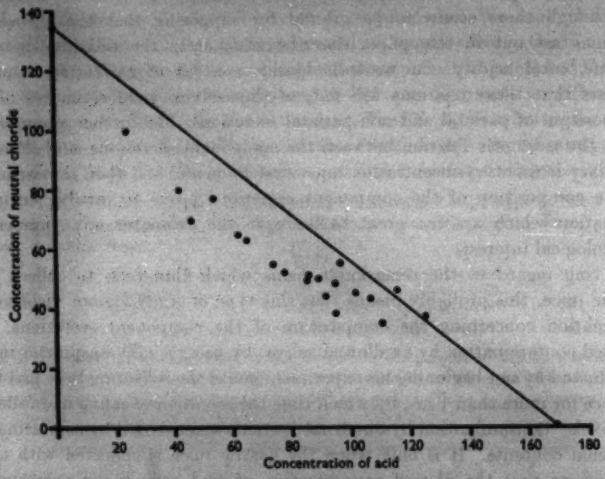

Fig. 3. The effect of omission of precautions on the relation between acid concentration and neutral chloride concentration. The points represent a series of observations by one of us (J.N.H.) on normal subjects stimulated with histamine. Salivary aspiration was used, but there was no duodenal suction and no waiting period before commencing collection of juice.

The estimate of y obtained is clearly dependent on the value of I assumed, but over the range of probable values of I this dependence is fairly small. Thus for $k_1 = 5{\cdot}44$, $k_2 = 9{\cdot}12$ (histamine juice) we have

$$y_{168} = 5{\cdot}44(N - 0{\cdot}162A), \quad y_{172} = 5{\cdot}44(N - 0{\cdot}113A).$$

That is, in this range, when A has a maximum value of around 30 m.equiv./hr., an error of 4 m.equiv./l. in the estimate of I will cause a maximum error in the estimation of y of about 8 ml./hr. Thus, if we take I as about 170 m.equiv./l., and write

$$y = 5{\cdot}44(N - 0{\cdot}14A),$$

then, for a sample of juice for which $A = 30$ and $N = 10$ m.equiv./hr., and $y = 31{\cdot}5$ ml., the error in the estimate of y due to inaccurate estimation of I is almost certainly within 10%. The absolute error similarly introduced into the estimation of the volume of parietal secretion will of course be the same, but the percentage error will be much smaller.

SUMMARY

1. The theoretical consequences of the assumption that the gastric juice is a mixture of two components of constant composition are developed.

2. It is shown that Ihre's (1938) extensive series of observations on human subjects fit the equations developed.

3. The relation of the equations developed to the various graphical methods for the determination of the composition of the component gastric secretions is demonstrated, and it is shown that an additional arbitrary assumption is involved in any such method.

4. Using the additional arbitrary assumptions: (*a*) that the component secretions have the same osmotic pressure, and (*b*) that the common cation concentration is 170 m.equiv./l., it is estimated that it is most probable that human 'parietal' secretion contains 160 m.equiv./l. of hydrion and 10 m.equiv./l. of neutral chloride.

5. It seems probable that the 'non-parietal' secretion elicited by histamine is of different composition from that elicited by insulin.

REFERENCES

Gilman, A. & Cowgill, G. R. (1933). *Amer. J. Physiol.* **103**, 143.
Gray, J. S. & Bucher, G. R. (1941). *Amer. J. Physiol.* **133**, 542.
Gray, J. S., Bucher, G. R. & Harmon, H. H. (1941). *Amer. J. Physiol.* **132**, 504.
Hollander, F. (1938). *J. biol. Chem.* **125**, 161.
Ihre, B. (1938). *Acta. med. Scand.* Suppl. no. 95.
Ivy, A. C. & Oyama, Y. (1921). *Amer. J. Physiol.* **57**, 51.
Katsch, G., Baltzer, F. & Brink, J. (1934). *Arch. Verdaukr.* **56**, 1.
Kozawa, S., Fukushima, K., Umeno, M., Kurihara, K., Takata, C. & Horiuchi, M. (1933). *Jap. J. med. Sci. VIII. Intern. Med.* **3**, 15.
Lifson, N., Visscher, M. B. & Varco, R. L. (1943). *Gastroenterol.* **1**, 784.

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THE ACCURACY OF THE MEYERSTEIN HAEMATOCRIT

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During the course of experiments to determine the degree of blood dilution by lymph, the inadequacy of established methods as an accurate index of dilution became obvious. The quantities of blood available were small, and a method involving not more than 50 cu.mm. of blood was essential. Using these quantities of blood none of the established photo-colorimetric methods for haemoglobin determination was accurate within ±2–5%, in our hands. Since the accuracy required was such that a difference of 2% was significant, red-cell counting was equally useless.

It was decided to investigate methods for the determination of red-cell volume. Probably the method of Wintrobe (1929) would have been sufficiently accurate, but required too much blood. The micro-method of Meyerstein (1942) was investigated, since it is now possible to make these haematocrit tubes from precision glass capillary tubing, and the volume of blood required is about 30 cu.mm. per tube. Experiments were performed to investigate the accuracy of the method and to determine the optimal conditions for its use.

METHODS

The apparatus has been described previously (Gilding, Meyerstein & Nutt, 1949). The haematocrit was made from Chance's precision glass capillary tubing, guaranteed internal diameter 0·8±0·01 mm. The lower end of the capillary was sealed in such a way that the end of the capillary was flat and at 90° to its length. The upper end was expanded into a cup of 0·4 c.c. capacity.

An instrument for accurate reading of the relative volumes of cells and plasma consisted of a Perspex holder for the haematocrit tube with an adjustable scale alongside. The scale, calibrated in 0·5 mm., lay in a slot at the side of the holder and was kept in a vertical position by a retaining spring; its lower end was attached to an adjustment screw. A double cursor running in two grooves at the sides of the holder had lines engraved on the front and back, thus avoiding errors in reading due to parallax. All readings were made with the aid of a lens.

Sets of tubes (usually eighteen in a set) were spun in a centrifuge of effective radius 15 cm., at speeds up to 6600 r.p.m., 5 min. always being allowed for the acceleration of the centrifuge. The relative volumes of cells and plasma were then read off by the aid of the measuring instrument, and the percentage cells calculated.

The relative centrifugal force applied to the blood column was calculated from the formula

$$\text{R.C.F.} = \frac{0{\cdot}0109}{980} n^2 mr,$$

where m = mass of blood = unity, n = r.p.m. and r = radius of centrifuge in cm. This is the force applied to the cell columns at the periphery. If the radius is measured to the centre of the column of packed cells it is 1·5–2·0 cm. less than that to the bottom of the cell column.

In these experiments rabbit, dog and human bloods were used.

Liquid paraffin was placed in the haematocrit cup to prevent evaporation, and blood was delivered under the paraffin from an approximately calibrated pipette. The volume of blood required was approximately 30 cu.mm. per tube, and the use of pipettes made from the same sample of capillary tubing as the haematocrit tubes prevented over-filling. Sufficient blood for several haematocrits could be obtained from a finger-prick. It was either collected into a small tube containing dry heparin or oxalate or measured directly in a heparinized pipette. Blood for the experiments designed to standardize the apparatus was collected from the ear veins of unanaesthetized rabbits or from the carotids of dogs anaesthetized with nembutal.

In some of the experiments the blood was withdrawn and stored under sterile conditions. To test the sterility, drops of the blood samples were cultured on to blood agar medium and incubated at 37° C. for up to 48 hr.; if no growth was evident the sample was assumed to be sterile. Samples for culture were taken at the beginning and end of each experiment.

RESULTS

Effect of Speed. In this series of experiments the time of centrifuging was kept constant at 30 min. and the speed and therefore relative centrifugal force was varied. Series of eighteen tubes were centrifuged at each speed from which the error of the method could be estimated by statistical analysis.

From Fig. 1 a typical experiment using oxalated rabbit blood, it can be seen that at speeds below 4000 r.p.m. in a 15 cm. radius centrifuge, i.e. at a relative centrifugal force below 3000 g. both the cell volume and the S.D. of each series decrease rapidly with increasing speed. Between this speed and 6000 r.p.m., i.e. a relative centrifugal force of 6000 g., the decrease of cell volume is not more than 1 %, while the S.D. remains constant between 0·2 and 0·3.

Effects of centrifugation speed on cell volume and standard deviation of each series. The results in Table 1 show that similar experiments confirmed these findings. Further experiments using oxalated dog blood were performed at speeds of 5000 r.p.m. and above. The S.D.'s of all series studied varied between 0·2 and 0·3.

These results suggest that 5000 r.p.m. in a 15 cm. radius centrifuge giving a relative centrifugal force of 4170 g. for 30 min. is the optimal force to be applied to the blood columns, though any force between 3000 and 6000 g. will not alter the rabbit cell volume by more than 1 %.

Effect of time. These experiments were done to determine whether the time of centrifuging might be reduced without increasing the error of the method. Earlier workers have shown that time of centrifuging is less important, though a time sufficient to obtain consistent results is desirable.

The results shown in Table 2 indicate the lesser importance of centrifugation time when speed is adequate, i.e. between 4000 and 6000 r.p.m. in a 15 cm.

radius centrifuge. The decrease in cell volume is less than 1% at any time of centrifuging up to 30 min., the S.D. varying between 0·2 and 0·3, 15 min. seems to be a suitable centrifugation time, allowing a preliminary 5 min. for the centrifuge to accelerate.

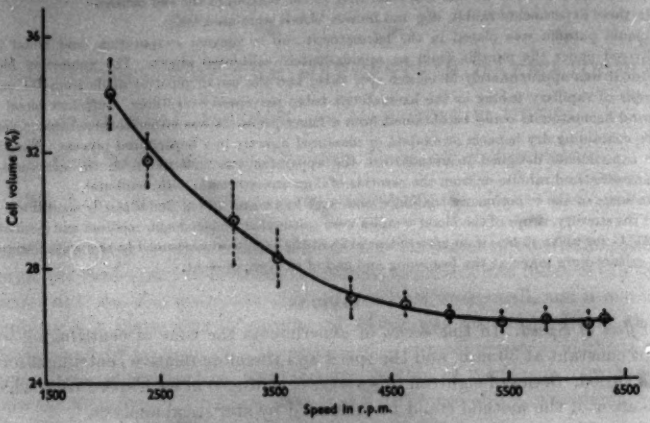

Fig. 1. Effect of speed on cell volume and S.D.

TABLE 1. Percentage cell volume

Blood	Below 2500 r.p.m.	2500–3000 r.p.m.	3000–3500 r.p.m.	3500–4000 r.p.m.	4000–4500 r.p.m.	4500–5000 r.p.m.	5000–5500 r.p.m.	Above 5500 r.p.m.
1	35·85 (0·67)	33·7 (0·56)	32·26 (0·41)	31·74 (0·30)	31·03 (0·21)	30·41 (0·22)	30·34 (0·41)	30·56 (0·22)
2	—	—	—	—	27·43 (0·26)	27·16 (0·20)	26·75 (0·22)	26·64 (0·18)
3	—	31·61 (0·40)	29·61 (0·23)	29·46 (0·27)	30·00 (0·26)	29·27 (0·23)	28·78 (0·24)	28·75 (0·14)
4	28·36 (0·45)	26·64 (0·37)	24·89 (0·42)	24·79 (0·35)	24·01 (0·11)	23·37 (0·11)	23·09 (0·20)	23·23 (0·15) 23·15 (0·19)
5	30·60 (0·43)	28·16 (0·33)	25·67 (0·16)	25·54 (0·29) 25·40 (0·27)	—	24·02 (0·18) 24·33 (0·19)	23·73 (0·18)	23·35 (0·17) 23·58 (0·14)
6	31·71 (0·46) 34·00 (0·60)	—	29·55 (0·69)	28·31 (0·49)	26·93 (0·31)	26·66 (0·18)	26·0 (0·16) 26·24 (0·11)	26·08 (0·09) 25·93 (0·13) 26·11 (0·17)

Figures in brackets = S.D. of series.

TABLE 2. Effect of time of centrifuging on cell volume and standard deviation (Centrifugation speed 5000 r.p.m. in 15 cm. radius centrifuge)

	5 min.		10 min.		15 min.		20 min.		25 min.		30 min.		35 min.	
Bloods	Vol. (%)	S.D.	Vol. (%)	S.D.	Vol. (%)	S.D.	Vol. (%)	S.D.	Vol. (%)	S.D.	Vol. (%)	S.D.	Vol. (%)	S.D.
Rabbit I	28·72	0·22	28·02	0·17	28·13	0·23	27·47	0·24	28·10	0·14	—	—	—	—
Rabbit II	37·99	0·26	37·31	0·12	37·34	0·21	37·0	0·12	37·11	0·18	36·95	0·25	37·13	0·27
Rabbit III	36·90	0·21	36·86	0·14	36·45	0·18	36·56	0·20	36·71	0·16	36·75	0·22	—	—
Dog I	50·79	0·24	50·6	0·28	50·04	0·22	50·26	0·19	50·35	0·24	—	—	—	—
Dog II	54·98	1·66	52·75	0·14	52·44	0·25	52·14	0·09	52·0	0·08	52·3	0·14	—	—
Dog III	—	—	—	—	—	—	52·96	0·22	52·96	0·21	52·61	0·21	—	—

Prevention of evaporation by liquid paraffin. Since evaporation during centrifuging is very considerable and the blood remains in the cup of the Meyerstein tube until centrifuging begins, it is essential to use some protection. It was decided to investigate the use of liquid paraffin for this purpose as had been done by previous workers (Smirk, 1928).

Oxalated rabbit blood was used for these investigations. Wintrobe haematocrit tubes, with and without a layer of paraffin, were centrifuged together with Meyerstein tubes. All the tubes contained samples from the same blood. A statistical analysis performed on the cell volume results showed that there was no significant difference between the results from the Meyerstein tubes and the Wintrobe tubes without paraffin or between the Meyerstein tubes and the Wintrobe tubes with paraffin. P in the t-tests performed lay between 0·1 and 0·2 in both cases.

Effect of storage. It had been realized that certain metabolic processes were continuing during the storage of the blood in the previous experiments,

TABLE 3. Effect of storage on cell volume

Storage temp. (° C.)	Storage time (hr.)	Cell volume (%)		
		Blood A	Blood B	
20	0	34·26	27·35	
	1	—	26·95	
	2	34·03	—	
	4	33·38	27·62	
	6	34·81	—	
	23	36·13	—	
	24	—	28·17	
	48	—	25·73	
	96	37·44	—	
		Blood C	Blood D	
37	0	31·16	26·79	
	1	31·71	—	
	3	32·71	—	
	5	33·67	—	
	6	33·04	—	
	8	34·68	—	
	9	34·97	—	
	12	35·06	29·15	
	13	—	28·76	
	14	—	28·94	
	15	—	27·66	
	16	—	25·86	
	17	—	26·76	
	19	—	25·32	
	20	—	12·93	
	25	23·71	—	
		Blood E	Blood F	Blood G
Sterile 37 storage	0	36·92	33·04	30·61
	6	39·71	35·94	—
	11	40·87	—	—
	16	—	—	24·38
	18	—	—	22·73
	19	—	—	20·86
	23	—	12·20	—

Baumberger (1942) had shown that leucocyte metabolic activity reduces oxyhaemoglobin in the layer of red cells adjacent to the buffy coat.

In these storage experiments heparin was used as the anticoagulant, since Guest & Siler (1934) had shown that it did not alter cell volume. All storage was undertaken in the darkness, for Meyerstein (1941) had shown that haemolysis is accelerated by light.

The results summarized in Table 3 show clearly that at a temperature of 20° C., the change in cell volume after 24 hr. storage is not more than 2 %. At 37° C. an increase in cell volume became apparent after 3 hr. storage, a maximum was reached after 12 hr., followed by a gradual decrease in cell volume. In a few blood samples this was followed by a sudden decrease of 10–12% of the cell volume.

Bacterial action seemed to offer a possible explanation of this sudden decrease in cell volume, therefore some further storage experiments, under bacteriologically sterile conditions, were undertaken. The results confirm those of previous storage experiments, bacterial action not being considered responsible for the storage haemolysis.

Accuracy of the method at clinical speeds. If the Meyerstein haematocrit was to be of any clinical value its accuracy at the speeds of a clinical centrifuge had to be investigated. It was assumed that the usual clinical centrifuge is of 10 cm. radius and has a maximum speed of 3000 r.p.m., which means it can exert a relative centrifugal force of 1000 g. at the periphery. The accuracy of the method under this condition, using normal heparinized human blood, was investigated.

Table 4. Accuracy of the method at clinical speeds (heparinized human blood)

	Speed...	5000 r.p.m.	2000 r.p.m.							
Subject	Time (min.)	15	15	30	45	60	75	105	120	150
1	% cells	43·30	44·80	44·40	44·30	44·12	—	—	—	—
	S.D.	00·10	00·33	00·34	00·24	00·36	—	—	—	—
2	% cells	38·04	—	—	—	39·05	—	38·83	38·74	38·54
	S.D.	00·25	—	—	—	00·28	—	00·31	00·26	00·20
3	% cells	37·50	—	—	38·25	38·17	—	—	—	—
	S.D.	00·11	—	—	00·21	00·18	—	—	—	—
4	% cells	37·04	—	—	—	37·76	37·63	37·57	37·55	—
	S.D.	00·29	—	—	—	00·20	00·30	00·28	00·17	—
5	% cells	36·96	—	—	37·77	37·60	37·52	37·37	—	—
	S.D.	00·11	—	—	00·18	00·24	00·33	00·12	—	—
6	% cells	36·14	37·06	36·94	36·81	36·67	—	—	—	—
	S.D.	00·29	00·14	00·18	00·23	00·35	—	—	—	—
7	% cells	35·29	37·04	36·49	36·46	36·36	36·45	—	—	—
	S.D.	00·35	00·40	00·38	00·27	00·31	00·32	—	—	—

From Table 4 it appears that a haematocrit value higher than that under the standard research conditions is obtained after centrifuging for times up to 150 min. After 45 min. there is no significant further decrease in the cell volume

or in the S.D. of a series. The accuracy of the method does not differ significantly from that obtained under the standard research conditions.

TABLE 5. Observed and calculated normal red-cell counts

Subject	Cell volume (%)	Observed R.B.C. × 1/10⁶	Calculated R.B.C. × 1/10⁶	Difference × 1/10⁶
		Males		
1	45·85	5·226	5·437	−0·211
2	45·49	5·140	5·396	−0·256
3	43·35	5·335	5·140	+0·195
4	42·10	4·547	4·992	−0·445
5	44·04	5·120	5·222	−0·102
6	45·34	5·069	5·254	−0·185
7	40·98	4·828	4·860	−0·032
8	45·69	5·378	5·415	−0·037
9	39·32	5·036	4·663	+0·373
10	40·30	5·015	4·779	+0·236
11	42·43	5·356	5·031	+0·325
12	42·88	5·215	5·083	+0·132
13	41·18	5·109	4·882	+0·226
14	44·91	5·300	5·325	−0·025
15	43·27	5·025	5·105	−0·080
16	40·08	5·095	4·752	+0·343
17	45·27	5·200	5·367	−0·167
18	40·20	4·852	4·767	+0·085
Mean	42·93	5·091		
		Females		
1	39·85	4·874	4·635	+0·239
2	38·25	4·761	4·450	+0·311
3	41·84	4·898	4·868	+0·030
4	37·90	4·011	4·408	−0·397
5	38·69	4·495	4·501	−0·006
6	39·01	4·455	4·538	−0·083
7	38·24	4·413	4·449	−0·036
8	37·43	4·140	4·354	−0·214
9	35·93	3·807	4·180	−0·373
10	37·77	4·593	4·393	+0·200
11	37·46	4·205	4·358	−0·153
12	38·46	4·430	4·478	−0·044
13	39·53	4·798	4·598	+0·200
14	35·93	4·330	4·180	+0·150
15	37·00	4·703	4·304	+0·399
16	36·75	4·355	4·276	+0·089
17	41·33	4·605	4·809	−0·203
18	39·20	4·568	4·560	+0·008
19	42·78	4·860	4·976	−0·116
Mean	38·59	4·489		

Normal human haematocrit values. A preliminary series of experiments has been done on male and female medical students and laboratory staff, in order to estimate the value of the normal adult cell volume and to investigate the possible substitution of this method for the red cell count. Capillary blood was collected into heparinized tubes and red-cell counts and Meyerstein haematocrits were done on each blood sample. The red-cell counts in Table 5 were estimated using a 'Thoma' Grid, each result is the mean of at least four counts on two separate blood dilutions.

The calculated red-cell count was found by averaging the observed red-cell count and the haematocrit for the whole series; then the calculated red-cell count was obtained from:

$$x \frac{\text{Mean observed red-cell count}}{\text{Mean haematocrit}},$$

where x is the individual haematocrit.

The mean corpuscular volume of men calculated from the mean haematocrit value and the red-cell count is $\frac{42{\cdot}93}{5{\cdot}091} \times 100 = 84{\cdot}3$ cu.μ. Similarly, for women the mean corpuscular volume is $\frac{38{\cdot}59}{4{\cdot}489} \times 100 = 85{\cdot}9$ cu.μ. These are within the accepted normal range of 82–92 cu.μ. So far these results indicate that the red-cell count calculated from the haematocrit does not show a significant difference from the observed red-cell value.

DISCUSSION

Earlier haematocrit methods had various disadvantages. Lengths of capillary tube, stoppered at one or both ends with metal or rubber caps were used (Hedin, 1891). Loss of blood during centrifuging was the important error here. These and other methods used, notably that of Wintrobe, involved graduation of individual tubes, and this was another source of unavoidable error. A disadvantage common to all these methods was the relatively large volume of blood necessary for the determinations. To overcome this latter difficulty micro-haematocrit methods were devised, the chief disadvantage of them all being the unequal bore of the capillary and the consequent error due to tapering of the tube. In the Hamburger tube, this error was taken into account by a preliminary calibration of a series of tubes using the same blood which was of known volume concentration. Smirk (1928) attempted to cancel the error due to taper by the use of a U of capillary tubing. In this tube the blood was protected by a layer of liquid paraffin and the relative volume of cells and plasma read off by placing the U on graph paper. The Meyerstein haematocrit reduces the above errors to a minimum. The capillary tubing used does not vary in diameter by more than ± 10 μ., and in most cases the variation is very much less than this. Since the volume of blood in each tube need only be measured approximately errors from this source are eliminated. Finally, the absence of graduations on individual tubes and the use of a simple measuring instrument reduces the cost of the apparatus, and eliminates this source of error.

In order to check the accuracy of the method the standard deviations of the results of all the experiments were calculated. At a relative centrifugal force of 3000 g. or above the S.D. remained constant between 0·2 and 0·3, which

means that 95% of the haematocrit results varying by more than ±2 S.D., i.e. 0·8–1·2% of the cell volume, are significantly different.

Similar analysis of the time results shows that at 5000 r.p.m. the S.D. lies between 0·2 and 0·3 over the time range 5–25 min.

5000 r.p.m. in a 15 cm. radius centrifuge, i.e. a relative centrifugal force of 4170 g. for 15 min., are suitable conditions for the use of the Meyerstein haematocrit. Under these conditions the accuracy of the method is ±0·6% of the cell volume in rabbit and dog bloods. However, there is some evidence that equally satisfactory results may be obtained on human bloods at slower speeds.

Using these standard conditions for the haematocrit estimations, the effects of storage on cell volume show that there is a definite small increase, due presumably to the accumulation of metabolic end-products. The practical importance of these results is to prove that blood samples may be measured into the Meyerstein tubes, under paraffin, and may be stored for 6 hr. at room temperature before centrifuging, without alteration of the cell volume.

As emphasized by Ponder (1948), the most common fault in published haematocrit estimations has been the failure to report radius and speed of centrifuge and time of centrifuging. This means that it is impossible to calculate the relative centrifugal force acting on the cell columns and therefore to compare results with those of other workers. The importance of the relative centrifugal force acting on the blood column was investigated by Millar (1925). He emphasized that haematocrit values were relative and dependent on the speed and radius of the centrifuge, and the time of centrifuging. Increase of the relative centrifugal force caused closer packing, until a point was reached when greater forces did not decrease the cell volume further. Millar claimed that the volume thus obtained represents the 'true' cell volume. Ponder & Saslow (1930) doubt whether it is possible to obtain a 'true' cell volume by the haematocrit method.

Unless the cells are completely packed, necessarily involving some distortion of their shape, some intercellular plasma must remain. This was shown by Maizels (1945), using a colorimetric method, to be approximately 2·7% of the volume of packed cells in human blood spun at 3000 r.p.m. in a 10 cm. radius centrifuge. No determinations of the intercellular plasma volume have been made in the present investigation. A report to the Traumatic Shock Committee of the Medical Research Council (1943) suggests that this does not remain constant in abnormal bloods so that no single correction factor may be introduced for any relative centrifugal force. It is possible that when great centrifugal forces are applied to the cell some of the intracellular water may leave the cell by a process of ultrafiltration. In theory this should not occur until the centrifugal force applied exceeds the osmotic pressure within the cell, i.e. is greater than 6·7 atm. This is equivalent to a relative centrifugal force of approximately 6900 g., and a speed of 6430 r.p.m. in a 15 cm. centrifuge.

Ideally, the relative centrifugal force applied to the blood column should be such that the mean corpuscular volume given by the haematocrit methods is the same as that of the cell suspended in its own plasma. Wintrobe (1946) states that when his tubes are spun at 3000 r.p.m. for 30 min. the volume of the red cell estimated by his haematocrit agrees exactly with that derived by Ponder by his colorimetric method, i.e. 87 cu.μ. In the experiments on human blood recorded above, the mean corpuscular volumes obtained were within the accepted normal range.

SUMMARY

1. The Meyerstein micro-haematocrit method is described using tubes of precision glass-capillary. Each determination only requires 30 cu.mm. of blood.

2. When Relative Centrifugal Forces of between 3000 and 6000 g. were applied the standard deviation was 0·2–0·3 for rabbit blood, i.e. the range of cell volume in any one series is not more than $\pm 0{\cdot}6\%$ from the mean cell volume. This corresponds to speeds of 4000–6000 r.p.m. in a centrifuge of effective radius 15 cm. Equally consistent results can be obtained for human blood at speeds as low as 2000 r.p.m.

3. The time of centrifuging under these high forces is much less important. From the results, 15 min. centrifugation at 5000 r.p.m. in a centrifuge of effective radius 15 cm. have been chosen as the optimal conditions for the use of the Meyerstein Haematocrit.

4. Storage of the blood samples at room temperature does not affect the cell volume for times up to 6 hr. after withdrawal from the animal. At 37° C. swelling of the cells is evident within 3 hr.

5. At clinical centrifugal forces, i.e. 2000 r.p.m. in a centrifuge of 15 cm. radius, the cell-volume determinations on human blood are about 0·5–1% greater than those estimated under standard research conditions, after centrifuging for 45 min. or more. The standard deviations of each series are of the same order as those under research conditions.

6. The red-cell count calculated from the haematocrit does not vary significantly from the observed red-cell count. The possible substitution of the haematocrit determination for the red-cell count, in individuals where the mean corpuscular volume is normal, seems worthy of further investigation.

REFERENCES

Baumberger, J. P. (1942). *J. Lab. clin. Med.* **27**, 1084.
Cox, R. T. & Ponder, E. (1941). *J. gen. Physiol.* **24**, 619.
Gilding, H. P., Meyerstein, W. & Nutt, M. E. (1949). *J. Physiol.* **108**, 32*P*.
Guest, G. M. & Siler, V. E. (1934). *J. Lab. clin. Med.* **19**, 757.
Hedin, S. G. (1891). *Skand. Arch. Physiol.* **2**, 134 and 360.
Köeppe, H. (1905). *Pflüg. Arch. ges. Physiol.* **107**, 183.
Maizels, M. (1944–6). *Quart. J. exp. Physiol.* **33**, 129.
Meyerstein, W. (1941). *J. Physiol.* **99**, 510.
Meyerstein, W. (1942). *J. Physiol.* **101**, 5*P*.
Millar, W. G. (1925). *Quart. J. exp. Physiol.* **15**, 187.
M.R.C. Report (1943). *Brit. med. J.* **1**, 209.
Ponder, E. (1948). *Haemolysis and Related Phenomena*. London: J. and A. Churchill Ltd.
Ponder, E. & Saslow, G. (1930). *J. Physiol.* **70**, 18.
Smirk, C. (1928). *Brit. J. exp. Path.* **9**, 81.
Wintrobe, M. M. (1929). *J. Lab. clin. Med.* **15**, 287.
Wintrobe, M. M. (1931). *Amer. J. clin. Path.* **1**, 147.
Wintrobe, M. M. (1932). *J. Lab. clin. Med.* **17**, 899.
Wintrobe, M. M. (1946). *Clinical Haematology*, 2nd. ed. p. 270. London: Henry Kimpton.

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AN ANALYSIS OF THE RESPONSE FROM SINGLE VISUAL-PURPLE-DEPENDENT ELEMENTS, IN THE RETINA OF THE CAT

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Hartline (1935) first showed that, on functional grounds, the optic nerve fibres of vertebrates could be divided into three main classes, according to whether they respond to the onset or the cessation of illumination or to both. He showed the existence of 'on-', 'off-' and 'on-off'-elements. Granit (1947), Granit & Tansley (1948) and Gernandt (1949) have studied the nature of such elements in the eyes of mammals, particularly of the cat, and have shown that elements may behave differently according to the wave-length and the intensity of the stimulating light. The recent work of Donner & Granit (1949), in which the light stimulus has been measured not so much in terms of absolute energy units, but in terms of its effects on the bleaching of visual purple, opens up new lines for investigation.

Comparison between the incidence of on-off-elements in the almost pure rod retina of the guinea-pig and their occurrence in the cat, in whose retina cones are more numerous, led Granit (1947) to the opinion that cones must participate in the production of the majority of the on-off-elements, and that the latter resulted from a stimulated element being antagonized or inhibited by another. Granit & Tansley (1948) came to a similar conclusion from studies of the 'off-on' ratio, and again considered that the inhibition was largely, though not exclusively, associated with cones. Donner & Granit (1949) have since shown that in the cat there are also 'on-off-elements' which act as if they are pure visual purple elements.

It is possible that off-effects may not only arise as the result of release from inhibition by another element; for it is theoretically possible that they could also arise, for example, from the effects of adaptation within a single cell or fibre. Against this idea, however, it has to be noted that certain elements which show adaptation quite strongly do not show any off-effects. Secondly, there are some elements which, at threshold, appear to depend only on visual

purple but which, at higher intensities, show off-effects, and these off-effects are sometimes more potent at the red end of the spectrum, even though the light stimuli are equal in terms of their effects on visual purple (equal V.P. stimulation). Such elements therefore must receive contributions from elements other than those dependent only on visual purple and it seems probable that, at least in these cases, a visual purple-dependent receptor is inhibited by another element which depends on some other photosensitive pigment, and which is probably a cone. The strong suggestion is, therefore, that off-effects do, in fact, mean interaction between two groups of cells and the occurrence of inhibition; but it would be a mistake in the present state of knowledge to lose sight of the other possibilities. For example, off-effects occur in the eye of *Pecten* (Hartline, 1937; Shoepfle & Young, 1936), though the probability of interaction between different neural elements appears to be somewhat remote on structural grounds. It may, however, occur. For the present, it will be assumed as a working hypothesis that off-effects are symbolic of inhibition of one element, or group of elements, by another, but it is not intended at this stage to suggest that all off-effects must be due to this cause. A reduction of the on-discharge, compared with the on-discharge of a pure on-element, particularly when coupled with the presence of an off-effect, will, similarly, be assumed to be evidence for inhibition.

The introduction of the equal V.P. stimulation simplifies the investigation of these elements which show off-effects, and the experiments reported in this paper were designed to study the nature of the response from those ganglion cells, or optic nerve fibres, in the retina which were activated directly or indirectly by receptors dependent on visual purple only. The results are of interest, partly in demonstrating the very great variation in the responses from different ganglion cells, partly in emphasizing that this variation may occur even though the photoreceptors themselves are all activated by visual purple and partly in providing further evidence that inhibitory processes are involved. Because of the very great differences between the responses from the ganglion cells, the results must be considered as indicative rather than exhaustive.

METHODS

All experiments were performed on isolated elements in the retinae of decerebrate cats, prepared by the standard technique (Granit, 1947). After decerebration, the cat was left in total darkness for about 90 min. so that apart from the effects of the test stimuli the retina was in the dark-adapted state and the preparation was in a fairly stable condition. After decerebration, 4–6 c.c. 20% urethane were given, to abolish small movements of the eye.

Each element, after isolation with the micro-electrode, was tested (equal V.P. stimuli) at $\lambda = 460$ mμ. and at $\lambda = 620$ mμ., sometimes also at $\lambda = 500$ mμ. and other intermediate wave-lengths. The intensities used were adjusted to have an equal bleaching efficiency on visual purple. For that purpose the curve determined by Donner & Granit (1949) for pure on-elements was used. The Wright colorimeter was used as the source of these monochromatic radiations. If the response was not demonstrably different at these different wave-lengths when tested at two or three

different levels of intensity (multiples of the equal V.P. units), then it was assumed that visual purple was the only photosensitive substance involved in the sensory layer. In the analysis of some of the elements, complete curves have been plotted from records obtained at several different wave-lengths and these were not significantly different. In these cases, therefore, there was certainly no material contribution made by any elements other than those sensitized by visual purple. Indeed for all the elements from which the data are discussed in this paper there was no evidence to indicate that anything besides visual purple was sensitizing the element or modifying its activity. Except in one or two series of experiments, each element was subjected to flashes of light lasting for 3 sec. The response as seen, after amplification, on the cathode-ray tube was photographically recorded for 1 sec. before illumination, for 3 sec. during illumination and for sufficiently long after the cessation of the stimulus for the majority of the after-effect, if any, to be recorded. The frequency of discharge of impulses in each 0·2 sec. period was then determined and the results plotted in graphical form. During the first 0·2 sec. period after both the onset and cessation of the stimulus the frequency changed very rapidly and the impulses were counted in 0·1 sec. intervals. The results are expressed as frequency per sec. The curves as shown have all been reduced by subtraction of the spontaneous discharge of the element under investigation, the spontaneous activity being determined before each test.

RESULTS

On-elements

In order to be able to determine whether or not, or to what extent, inhibitory processes are contributing to the response from an isolated element, it is necessary first to study those elements which give pure on-discharges, as these are almost certainly the simplest type of element, and, in them, interference from other elements is probably minimal.

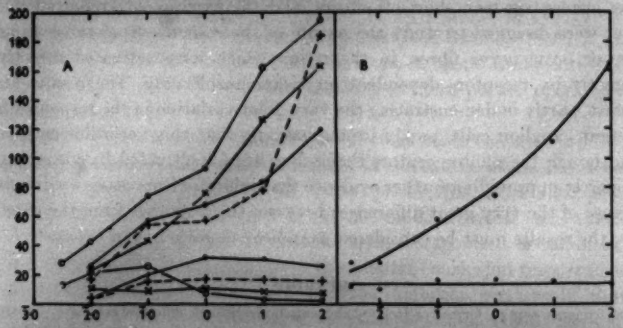

Fig. 1. Relationship between the frequency of impulses during the on-discharge and the light intensity in four different elements. Ordinates: impulses per sec. Abscissae: light intensity in logarithmic units. Upper curves: initial discharge. Lower curves: discharge after 3 sec. A, individual elements; B, mean values.

The responses from four such elements have been recorded and the following conclusions can be drawn. The magnitude and the character of the response vary with the intensity of the stimulus, but with equal V.P. stimuli, they are independent of wave-length. The initial outburst of impulses when the stimulus

is applied shows a frequency which varies almost linearly with the logarithm of the intensity of the stimulus (Fig. 1). After 0·2–0·3 sec., however, the frequency declines rapidly, and at the end of the 3 sec. period of stimulation is much more nearly independent of the intensity of the stimulus, though the elements differ to some extent in the magnitude of this delayed or 'equilibrium' response. A stimulus lasting only 3 sec. is rather short and may not allow a precise determination of the asymptote and so of the exact value of the 'equilibrium' response. Moreover, although some elements may behave as pure on-elements at low intensities, they often show a change of character at high intensities, and the final response at high intensities may be partly determined by inhibitory processes. Certainly, in some cases an off-effect may suddenly appear at high intensities. Fig. 2 shows the frequency of response from one element at five different intensities and also the final phase of the response from another similar on-element at a relatively high intensity. This last clearly shows an off-effect.

It seems likely that the rapid decline in frequency while the stimulus is maintained is more of the nature of an adaptation process than one caused by inhibition from neighbouring elements, but in a complicated structure like the cat's retina the possibility of inhibition cannot be eliminated. In general, the higher the initial frequency the more rapid is the decline, and it may be of interest to observe that in the element for which the frequencies are recorded in Fig. 2 the area enclosed by the curve depicting the frequency during the on-discharge is almost identical for the three highest levels of intensity. It is as though a maximum amount of activity can be evoked, and if this activity

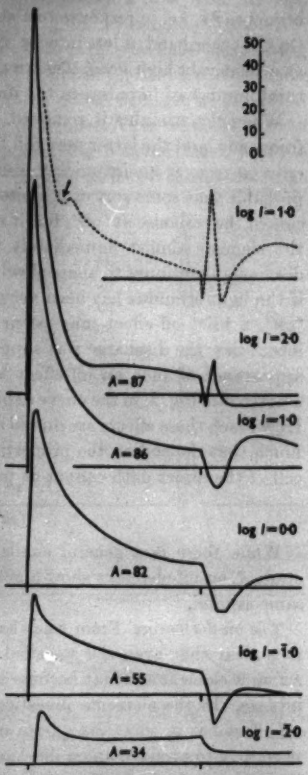

Fig. 2. Impulse frequency in the discharge of on-elements at different light intensities. Ordinates: impulses per sec. Abscissae: time; the duration of the stimulus being 3 sec. The continuous curves are all from the same element; the dotted curve is from another element. *A*, area enclosed by the curve representing the on-discharge. The arrow indicates the beginning of a notch in the curve which in some elements becomes, at high intensities, much larger than the one shown.

occurs early, i.e. in response to a strong stimulus, then it must decline quickly. On the other hand, a less intense initial discharge can be maintained for longer at a relatively high level. The area within the curve, of course, represents the total number of impulses in the discharge.

When the stimulus is removed, there is generally an immediate decline in frequency, and the latter may fall below the level of the spontaneous discharge, only to recover again quickly and then slowly decline once more. It seems probable that some sort of inhibitory influence is at work to cause this depression of the response at 'off', but it must clearly be of a transitory nature so that the element almost immediately begins to discharge again and the 'after-discharge' continues to abate slowly. As already indicated and shown in Fig. 2 if the light stimulus has been very strong then, instead of a 'silent' period at 'off', a brief off-effect may occur within the period during which, at lower intensities, the discharge was suppressed at the cessation of the stimulus. The appearance of such an off-effect is sometimes correlated with appearance of a notch (see Fig. 2) in the curve depicting the frequency during the on-discharge. How much these effects are due to inhibitory processes from other cells and how much they depend on the properties of the receptor, bipolar cell and ganglion cell of the direct path cannot at present be determined.

On-off-elements

While there is a general similarity in behaviour of the four on-elements studied, on-off-elements show much greater differences in their responses to the same stimuli.

The on-discharge. From what has been said in the section on 'on-elements', it is clear that even the so-called pure on-element is by no means certainly a simple element and may become an on-off-element if the stimulus is sufficiently intense. In the elements investigated the necessary intensity to produce an off-effect has in some cases been more than one thousand times the intensity necessary to produce an on-discharge. Possibly even higher values may sometimes be required. On the other hand, Fig. 3 A shows an element which at low intensities gives a pure on-effect without even an after-discharge, but whose response is converted into a definite on-off type by raising the intensity of the stimulus only one hundred times.

Furthermore, elements which begin as on-off-elements at low intensities may have their on-discharges entirely suppressed at higher levels and so become off-elements (Fig. 3 B). It appears therefore that the differences between on-, on-off-, and off-elements are largely determined by the balance set up in the elements by excitatory and inhibitory processes. As the latter becomes more prominent so the off-effect becomes more pronounced. In a typical on-off-element, and while the intensity of the stimulus is low, the on-discharge increases progressively with intensity at about the same rate as that of a pure

on-element, but, as the intensity is raised, there comes a time when the on-discharge ceases to increase and may even fall (Fig. 4). Presumably the depressant effects of inhibition becomes greater at that point than the augmenting effects of the direct stimulus. Although the inhibitory process is eventually effective in preventing an increase or causing an actual decrease in the initial on-discharge it gives the impression of being more effective in reducing or even eliminating the delayed or 'equilibrium' response. Fig. 5 shows clearly how the delayed response is reduced at the higher intensities while the initial discharge

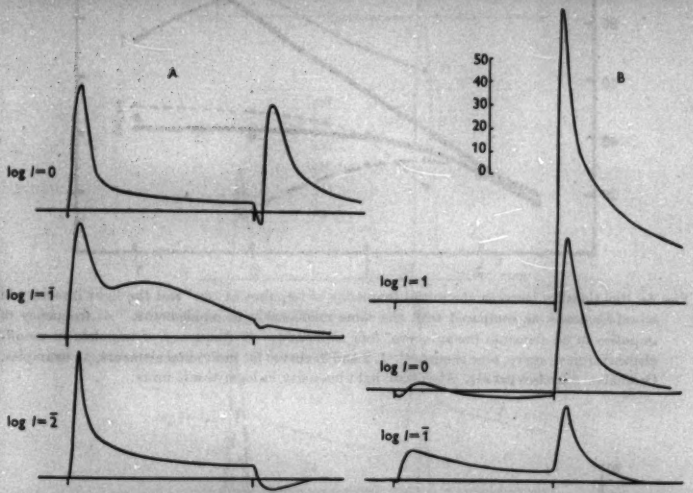

Fig. 3. A, discharge of an element which changes from the 'on' type to the 'on-off' type as the intensity of the stimulus is raised. B, discharge of an element which changes from the 'on-off' type to the 'off' type as the intensity of the stimulus is raised. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec.

may be still on the increase. The point, however, is difficult to assess since the delayed response of a pure on-element becomes relatively and sometimes absolutely less important the higher the intensity of the stimulus. As a result of this effect of inhibition on the delayed response, some on-off-elements only discharge for a short time at 'on' and then again at 'off', with a long silent period while the stimulus remains in action (Figs. 5, 6).

An attempt has been made to analyse how the inhibitory process acts on the on-discharge by comparing the average discharge of several on-off-elements at different intervals after the onset of the stimulus, and at different levels of light intensity with the corresponding discharges in 'pure' on-elements. The highest

intensities have not been used because, with these, the so-called 'pure' on-elements may suffer some degree of inhibition. Since there is very wide varia-

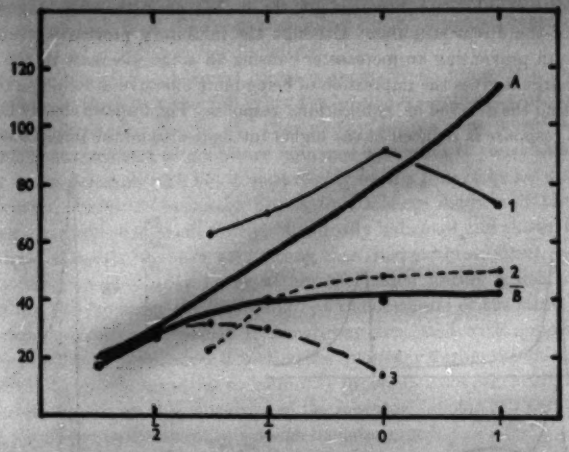

Fig. 4. Relationship between the initial frequency of impulses at 'on' and the light intensity in on-off-elements, as compared with the same relationship in on-elements. *A*, frequency of impulses in on-elements (mean curve, four elements). *B*, frequency of impulses in on-off-elements (mean curve, nine elements). 1, 2 and 3, curves for individual elements, as examples. Ordinates: impulses per sec. Abscissae: light intensity in logarithmic units.

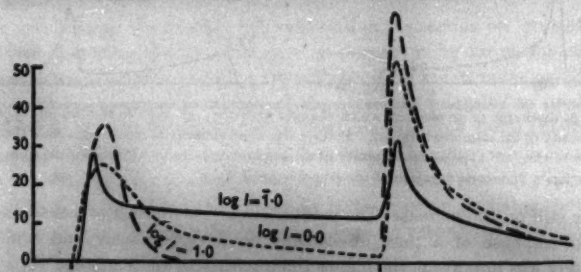

Fig. 5. Impulse frequencies at three different intensities of the light stimulus in an on-off-element showing how the delayed response may be completely inhibited while the initial discharge remains. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec.

tion in the responses from the on-off-elements, probably indicating different proportions of inhibition to stimulation, the pooling of results is unsatisfactory, but is unfortunately the only way of treating the data. Although the actual

numerical values obtained are probably of little significance the results seem to indicate fairly clearly the sort of way in which the inhibition counteracts the

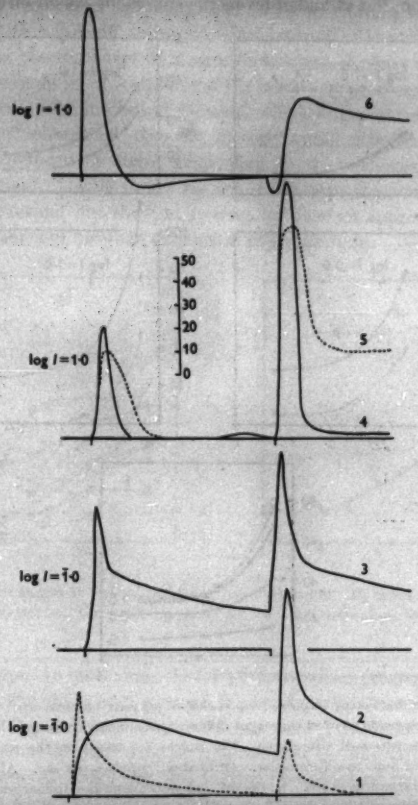

Fig. 6. Various types of discharge from on-off-elements. 1, 2 and 3 at intensity 1·0; 4, 5 and 6 at intensity 1·0. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec. Note the delayed on-discharge in 2, which corresponds with a type of discharge described by Hartline (1935) for the frog. Note also the character of the off-discharges in 4 and 6.

excitation. The continuous curves in Fig. 7 A–D show the mean frequency of response from the pure on-elements 0·2, 0·8 and 1·6 sec. after the onset of the stimulus and at four different intensities. The dotted curves show the mean

frequencies of response for the on-off-elements under the same conditions. At the lowest intensity both curves are much the same, and there is no evidence for any inhibition, but at higher levels the curves for the on-off-elements are all

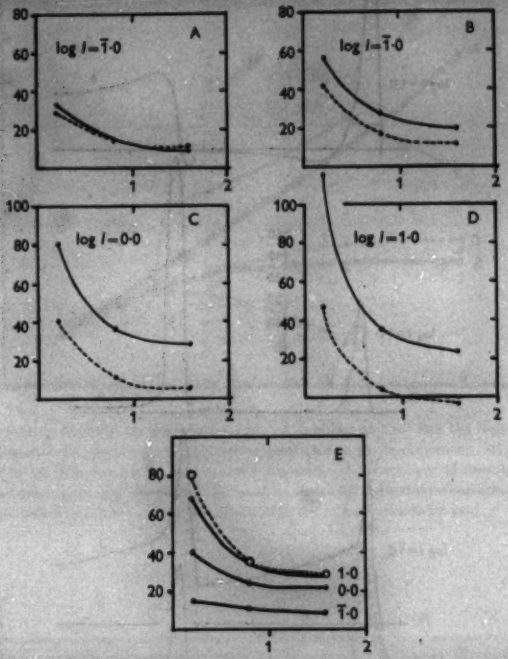

Fig. 7. A–D, curves indicating impulse frequencies of (*a*) pure on-elements (continuous curves), and (*b*) on-off-elements (dotted curves) at different time intervals during the on-discharge and at different intensities of stimulation. The curves are based on the mean values for four on-elements and nine on-off-elements. Ordinates: impulses per sec. Abscissae: time after the onset of the stimulus in sec. E, the differences between the discharges of the on-elements and those of the on-off-elements in B, C and D, as measures of the amount of inhibition which occurs at the three higher intensities, are plotted as continuous curves. The dotted curve represents the mean response of the four on-elements at the same time intervals after stimulation and at intensity 0·0. The amount of inhibition at intensity 1·0, thus corresponds closely to the response of an on-element to a stimulus of one-tenth the strength.

lower, and indicate a reduction or inhibition of the on-discharge. The extent of the inhibition increases steadily with the intensity of the light and acts to depress the magnitude of the response throughout its whole time course, the initial response being actually depressed rather more than the delayed response,

especially at higher brightnesses. When the differences between the 'on' curves and the 'on-off' curves of Fig. 7 are plotted, the resulting curves (Fig. 7 E) may be considered to represent the magnitude of the inhibition which has taken place. The inhibition falls off during the time interval of the stimulus at much the same rate as the discharge of a pure on-element declines under the same conditions, so it would seem possible that the inhibitory process may be a purely subtractive process; the inhibiting element, which in general responds less intensely than the 'stimulated' element, directly antagonizes the latter so that its response is reduced in direct proportion to the response of the former (inhibiting) element. Owing to the nature of the data these results should be regarded with caution, but they do at least indicate an approach which may clarify the relationship between excitation and inhibition.

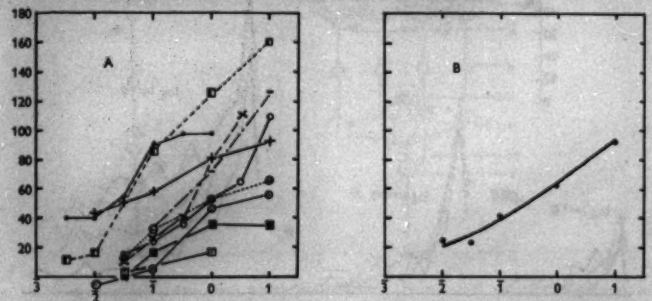

Fig. 8. A, initial discharges at 'off' in individual on-off-elements. B, mean initial discharge at 'off' in on-off-elements. Ordinates: impulses per sec. Abscissae: logarithm of the intensity of the stimulus.

The off-discharge. In most cases the off-effect increases progressively with the increase in intensity of the light in much the same way as the on-discharge of an on-element (cf. Fig. 8 B and Fig. 1 B). At very high light intensities the maximum frequency at 'off' may be reduced and the discharge may become delayed or prolonged. There are also further complicating factors in the off-discharge, for in some elements the discharge is sudden and rapidly dies down again, while in other elements it develops a conspicuous 'tail' which becomes higher and longer, the greater the intensity of the original stimulus. In this connexion the results of two experiments may be mentioned, as they may be important in the interpretation of off-effects in general though, since only two elements have so far been examined from this point of view, the results are mentioned with some diffidence. It was found that at one level of light intensity (about 100 times threshold) the duration of the stimulus between the limits of 2 and 5 sec. made no significant difference to the size or duration of the off-

effect. At one-tenth of this intensity the off-effect was rather small, and the duration of the stimulus only contributed rather doubtfully to its character. If there was any effect at all, then the longer the duration of the stimulus the less intense the off-effect. Probably the off-effect here was more in the nature of an after-discharge, and the longer the duration of the stimulus the more adaptation has taken place and so the smaller the after-discharge. On the other hand, when the intensity of the stimulus was high (about 1000 times threshold) the size of the off-effect definitely increased with the duration of the stimulus. Both the immediate discharge was somewhat greater and the 'tail' was conspicuously increased and prolonged (Fig. 9). In another element, in

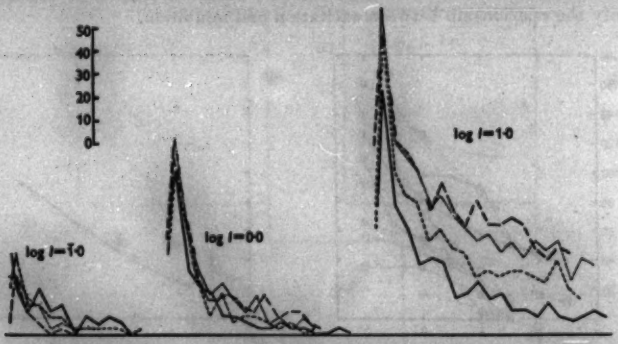

Fig. 9. The off-discharge of an on-off-element at three different intensities, showing the effect of the duration of the stimulus in increasing and prolonging the discharge at the high intensity only. Duration of stimulus: ——, 2 sec.; - - - -, 3 sec.; . . . ., 4 sec.; – – – –, 5 sec.

which the tail of the off-effect was almost absent, the duration of the stimulus had practically no effect, at any intensity level, on the character of the off-discharge.

DISCUSSION

The first point to be emphasized on examination of the records discussed in this paper is the great variation and complexity of the elements, for it must be remembered that all the receptor elements which contribute to the picture under discussion are activated by visual purple only, i.e. the receptors belong to the rod family only, and there was no evidence that any of the elements analysed behaved differently at different wave-lengths as they would do if cones were contributing to the effects. The stimulating lights, when of different wave-lengths, were always such as to cause equal stimulation of receptors dependent on visual purple only, so that there is no need to assume that any other than visual purple-dependent receptors were involved.

The elements whose electrical behaviour was under investigation were probably ganglion cells or their fibres and it is important to remember that between the receptor itself and the ganglion cell there are, at the very least, two synapses (see Fig. 10 A). The actual arrangement of the cells and their synaptic connexions is represented diagrammatically in Fig. 10 B, which itself is probably a great over-simplification of the real state of affairs. At present it is impossible to attribute any of the effects observed to particular elements in this complex, except the initial photoreception to the rods, conduction to the bipolar cells and

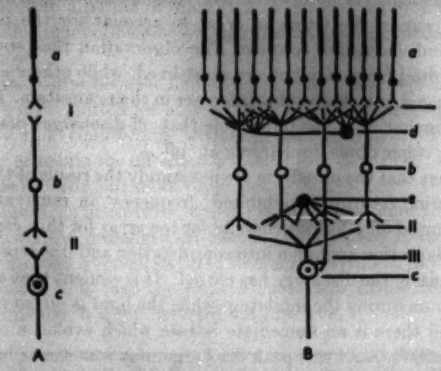

Fig. 10. The structural basis from which the impulses are recorded. A, the simplest possible system. The electrode picks up discharges from *c*. B, diagram showing the main groups of cells which may be concerned. Again the electrode picks up from *c*. *a*, Rod; *b*, bipolar cell (probably of more than one type); *c*, ganglion cell (probably of more than one type); *d*, horizontal cell; *e*, amacrine cell; I, synapses between rods and bipolar cells; II, synapses between bipolar cells and the dendrites of ganglion cells; III, synapse between bipolar cell and the cell body of a ganglion cell.

the observed electrical discharges to the ganglion cells or their fibres. Comparison with results from the eye of *Limulus*, as recorded by Hartline & Graham (1932), indicate that some of the effects may be inherent in the receptor or, at most, in a single synapse. For example, the initial discharge, followed by a period of adaptation, is exactly comparable with the discharge in the fibres of *Limulus*, including even the 'notch' which develops at high intensities immediately after the initial outburst of impulses and which in the elements in the cat's retina seems to be associated with the appearance of an off-discharge. Practically none of the ganglion cells observed in these experiments has ceased to discharge immediately after the light has been turned off and has then remained 'silent'. There is generally a momentary pause of so-called post-excitatory inhibition (Granit, 1944) in the discharge at 'off', followed by what

appears to be an after-discharge. Are these changes observed at 'off' to be considered as properties of the receptor, or the synapses, or are they caused by some actively inhibiting agent or elements?

Off-effects proper are probably the result of inhibition. In the examples discussed here they must be caused by an element or elements sensitized by visual purple. Such may be identical with or include the same element as is causing the initial on-discharge, but presumably working along some parallel pathway or, alternatively, neighbouring elements may be involved. The latter suggestion is decidedly the more attractive, since some such mechanism may be presumed to occur in the retina in order to account for the phenomena of simultaneous and successive contrast. The observation that some off-effects attain a high initial frequency but are short-lived, while others are less severe but more prolonged, may indicate a difference in their causation. To complicate the issue still further, it is not impossible that off-discharges may themselves be inhibited by other fibres discharging at 'off'.

It is quite clear that the off-effects are not simply the result of the stimulated element recovering its own 'uninhibited' frequency on removal of the light stimulus. The initial off-effect is generally far too great for this, especially when the effects of adaptation are taken into consideration and it is remembered that the stimulus causing the discharge has ceased. It is evident that some cumulative process occurs during the inhibition while the light is on, so that when the light is removed there is an immediate release which evokes a corresponding high rate of discharge, and this peak discharge may sometimes be followed by a very prolonged 'tail' discharge or period of activity which only gradually dies away. When the light stimulus is intense enough, both the peak and the tail may be increased by prolonging the period of illumination, as if some chemical transmitter had accumulated while the period of inhibition was in progress, or as if the inhibiting adjacent element itself had suffered inhibition and thus ceased to influence the fibre picked up by the electrode. It is important, however, to note that the duration of the stimulus does not necessarily affect the character of the off-effect.

It will be interesting to compare this picture of stimulation and inhibition, which is dependent only on receptors sensitized by visual purple (rods), with that which occurs when elements other than those dependent on visual purple (cones) are involved.

SUMMARY

1. Impulse frequencies have been recorded from single elements (probably ganglion cells) in the retina of the decerebrate cat. All elements selected were sensitized by visual purple only.

2. A single element may behave first as an on-element, then an on-off-element and finally as an off-element depending on the strength of the stimulus used.

3. Different elements show responses which suggest different degrees of excitation and inhibition. Since the least inhibited elements, the so-called pure on-elements, show an initial outburst of impulses followed by rapid adaptation it is difficult to determine when inhibition proper begins to exert its effects. The appearance of a peak off-effect has generally been taken to indicate inhibition.

4. Inhibition reduces both the initial and the delayed frequency of impulses during the on-discharge. The effect is actually greater on the initial outburst but is relatively greater on the delayed discharge.

5. There is often a period of inhibition or reduced activity at 'off' in those elements which might otherwise be regarded as pure on-elements. An after-discharge follows.

6. The relationships between light intensity and peak frequency at both 'on' and 'off' have been plotted for both on- and on-off-elements, and also between light intensity and 'equilibrium' frequency (at 3 sec.) in on-elements.

7. In some elements the off-effect is intense and shortlived; in others it may be less intense initially but more prolonged.

8. With high light intensities, increase of the duration of the stimulus may lead to increased and prolonged off-effects, but does not necessarily do so.

9. Maximum peak frequencies up to 200 impulses per sec. have been recorded, but owing to the fact that the camera speed was rather too low for these frequencies, this figure is put forward with reserve.

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REFERENCES

Donner, K. O. & Granit, R. (1949). *Acta physiol. Scand.* **1**, 161.
Gernandt, B. (1949). *Acta physiol Scand.* **17**, 150.
Granit, R. (1944). *J. Physiol.* **103**, 103.
Granit, R. (1947). *Sensory Mechanisms of the Retina.* Oxford University Press.
Granit, R. & Tansley, K. (1948). *J. Physiol.* **107**, 54.
Hartline, H. K. (1935). *Amer. J. Physiol.* **113**, 59.
Hartline, H. K. (1937). *Amer. J. Physiol.* **119**, 328.
Hartline, H. K. & Graham, C. H. (1932). *J. cell. comp. Physiol.* **1**, 277.
Schoepfle, G. & Young, J. Z. (1936). *Biol. Bull. Woods Hole*, **71**, 403.

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THE RESPONSE OF THE RABBIT'S ENDOMETRIUM TO UTERINE IMPLANTS OF PROGESTERONE AND OTHER STEROIDS

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The progestational proliferation reaction of the rabbit's endometrium to injected progesterone has long been used as a method of assay for this substance. Attempts to make the method more sensitive by applying the hormone directly to the responding tissue have been made by McGinty, Anderson & McCullough (1939), who introduced oily solutions of the substance to be tested into the uterine lumen of immature rabbits, pre-treated with oestrogen. Responses to doses much smaller than those required when progesterone is given systemically, are obtained in this way. However, the lack of uniformity of response among different animals makes this technique somewhat unsatisfactory for purposes of quantitative assay.

It appeared that introduction of the hormone in solid form into the uterine lumen might produce more uniform results, and this has been the basis of the method used in the experiments reported here. Such solid implants, in doses smaller than those necessary to produce an effect when given systemically, were found to produce an endometrial reaction confined to the segment of uterine horn implanted. The response thus follows the *direct* and *local* action of the implanted hormone. This type of action in the case of progesterone appears to form a satisfactory basis for a method of assay. Since steroids, other than progesterone, have a progesterone-like action when given systemically, the ability of certain steroids to produce the direct local response has also been investigated and compared with their systemic effect.

METHODS

Mature, ovariectomized rabbits were used. For 7 days before implantation they were given a daily subcutaneous injection of 10 μg. of oestrone in olive oil. On the day following the last oestrone injection the uterus was exposed by midline incision. An opening was made with scissors into the horn and the implant inserted and placed at least half a centimetre away from the opening. Implants were held in place by ligatures encircling the uterine horn above and below the implant.

A number of vessels pass from the mesentery into the uterus and no difficulty was encountered in ensuring that the implanted segment received an adequate blood supply from at least one arterial vessel in the area between the two ligatures. The length of the uterine horns is such that two implants could be placed, utilizing a common opening, one towards the ovarian, the other towards the vaginal end of each horn. Thus four tests could be carried out in any one animal. The implants were normally inserted with forceps but, if very small, they were placed in the mouth of a narrow glass tube and extruded by pushing them out with a well-fitting glass rod acting as a piston. The opening in the uterine horn was closed by a purse string suture.

The experimental animals were killed 4 days after implantation. The implanted segments and a control segment were excised and fixed in formalin. Paraffin sections were made and stained with haematoxylin and eosin. On microscopic examination the progestational response was rated as from 1 to 4, using the semi-quantitative measurements of degree of response described by McPhail (1935). Preliminary experiments showed that the mere isolation of a segment between ligatures, or the implantation of cholesterol pellets (since some of the substances were given as mixtures in cholesterol), produced no changes in the endometrium. Hence, any unimplanted portion of the uterine horn could serve as a control. In most cases absorption of the implant was not complete after the routine 4-day period and residues were therefore looked for at the time of removal of the implanted segments. All residues recovered were placed in a desiccator for a few days to dry and then weighed.

RESULTS

The direct local response to implants of 1% *progesterone in cholesterol*

Preliminary experiments with implants of crystalline progesterone were made, but the minimum effective dose in these cases was still somewhat high. It seemed probable that this might be due to excessively rapid absorption of the implants. Shimkin & White (1941) have shown that the absorption rate of steroid hormones from implanted pellets is greatly decreased when pellets containing hormone mixed with cholesterol are used. Therefore, subsequently the implants consisted of a fused mixture of 1% progesterone in cholesterol. The rate of absorption from such implants was satisfactory.

TABLE 1. Response to uterine implants of 1% progesterone in cholesterol

Progesterone (μg.)	No. of animals	Grade of response (McPhail, 1935)	Implant weight	
			Original (mg.)	Average residual (mg.)
100	3	3, 4, 4		
50	3	4, 4, 4	5	3·55
25	12	3, 4, 2, 4, 2, 2, 3, 4, 4, 3, 4, 4	2·5	2·2
20	13	4, 3, 4, 2, 3, 3, 3, 4, 4, 3, 3, 4, 4	2	1·6
10	12	2, 2, 3, 4, 4, 4, 3, 4, 3, 1, 4, 3	1	0·9
5	12	3, 1, 2, 4, 3, 3, 3, 2, 1, 1, 2, 2	0·5	0·36
4	10	3, 2, 1, 2, 1, 2, 2, 0·5, 0·5–1, 0·5–1	0·4	0·4
3	10	0·5, 2, 3, 2, 1, 1·5, 1, 2, 1, 0·5–1	0·3	0·3
2·5	3	3, 0, 2	—	—
2	12	3, 4, 0, 2, 1, 0, 1, 2, 1·5, 2, 0·5, 1·5	0·2	0·22
1	13	2, 0, 0, 0, 1, 1, 2, 1, 1, 0, 0·5, 0, 0	—	—
0·5	10	0, 0, 0, 0, 0, 0, 0, 0, 0, 0·5	—	—
0·2	2	0, 0	—	—

Table 1 shows the responses obtained with different amounts of progesterone given in such implants, as well as the average weight of the residues where a sufficient number were recovered. It will be seen that the minimum effective

dose of progesterone under these circumstances is about 1 μg. In Fig. 1 some of the data from Table 1 are used to construct the curve of the percentage of

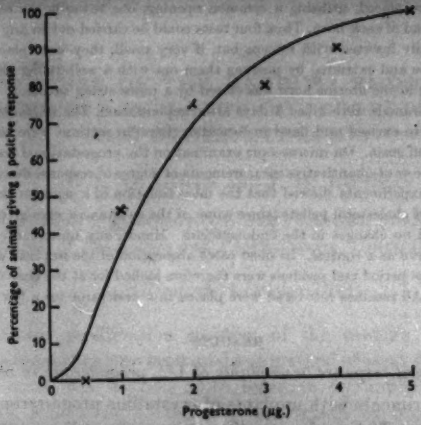

Fig. 1. Curve relating percentage of animals giving a progestational response rated as at least 1 (McPhail, 1935) to uterine implants of 1% mixtures of progesterone in cholesterol.

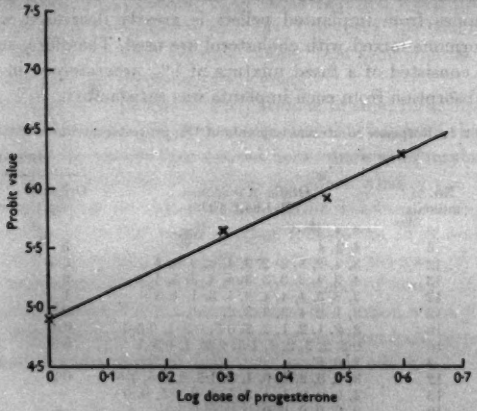

Fig. 2. Probit log dose regression line for doses of progesterone from 1–4 μg. given as uterine implants of 1% mixtures in cholesterol.

animals giving a response rated as at least 1, for the critical range of dose values. A sigmoid response curve is obtained. In Fig. 2 the response to doses

over the range 1–4 μg. is shown as a probit regression line. This regression line, and calculations based on it, using the methods of Finney (1947), give a median effective dose of $1{\cdot}08 \pm 0{\cdot}06$ μg.

Using these data, it should be possible to assay the amount of progesterone in implants prepared from extracts to which cholesterol has been added. Further work on these lines is in progress.

Consideration of the residual weights of the recovered implants in Table 1 shows that during the 4-day period only a part of the implants was absorbed. In the case of the smaller implants of 0·2–0·4 mg. no decrease in weight was shown, and in individual cases there was occasionally an increase in weight. This is believed to be due to deposition of variable amounts of endometrial exudate on the implant. In the case of small implants this appeared to mask the loss of weight due to absorption of material from the implant. At any rate, it is clear that the minimal amount of the hormone which has to pass into the endometrium to produce a progestational effect is less than 1 μg.

TABLE 2. Response to uterine implants of 1% progesterone in cholesterol in animals not primed with oestrogen after ovariectomy

Dose (μg.)	Percentage of primed animals showing response (>0·5)	Grade of response in unprimed animals at various periods after ovariectomy (days)						
		6	7	11	12	33	35	36
20	100	—	—	—	—	1–2	3–4	2
10	100	2	2–3 2	—	1·5	2	1–2	2
5	100	2	—	2	—	0	0·5	0·5
4	90	— —	0·5–1 0·5–1	—	0 —	— —	— —	— —
3	80	—	—	—	—	0	0	0·5
2	75	—	0	0·5	0	—	—	—
1	46	—	0	—	0	—	—	—

Since in experiments with uterine implants progesterone is absorbed directly into the endometrium, it is possible that under these circumstances preliminary treatment with oestrogens might be unnecessary. Experiments were, therefore, made in which ovariectomized rabbits were kept for varying periods after ovariectomy and were then implanted without any preliminary oestrogen injections. The results of these experiments are given in Table 2. It is seen that when the animal was deprived of oestrogen for 11 days the minimal effective dose of progesterone was above 2 μg., while in two animals deprived of oestrogen for about a month this value was above 5 μg. This shows that even when progesterone is directly administered to the endometrium its sensitivity depends on the previous action of oestrogen. The results are also in conformity with the findings of Hisaw, Greep & Fevold (1937) and Selye (1940) who, working with systemic injections of progesterone, found that progestational responses can be obtained after considerable periods of oestrogen deprivation, but that much larger doses of progesterone are required.

The direct local response to steroids other than progesterone

The results of experiments with uterine implants of other steroids either as the pure substance in the form of fused crystals or as a powder, or as mixtures in cholesterol as pellets or as a powder, are shown in Table 3. The last column indicates whether or not the substance in question has been shown to have progesterone-like activity on the rabbit's endometrium when given by systemic injection. This information is based on the findings of Selye & Masson (1943) and Klein & Parkes (1937).

TABLE 3. Endometrial response to steroids other than progesterone

Substance	Amount introduced in implanted segment	Response: Implanted segment	Response: Control segment	Residual weight of implant mg.	Remarks	Response when injected systemically
Ethisterone	2 mg. I	3–4	0	—	2 % in cholesterol I	Active
	0·68 mg. I	4	0	0·61		
	0·3 mg. I	2	0	—		
	0·2 mg.	4	0	17·4	1 % in cholesterol I	
	0·1 mg.	4	—	8·3		
	0·02 mg.	3	—	—		
	10 μg.	2	0	1	1 % in cholesterol I	
	5 μg.	2	—	0·17		
	2 μg.	1·5	—	—		
	1 μg.	1·5	—	—		
	10 μg.	2–3	0	0·8	1 % in cholesterol P	
	5 μg.	1–2	—	—		
	3 μg.	1–2	—	0·3		
	2 μg.	0·5–1	—	0·4		
Pregnenolone	20 mg. I	3–4	Not done	16·5	—	Active
	5 mg. I	3–4	Not done	5·4	—	
	1 mg. I	3–4	1	0·6	—	
	0·1 mg. I	0	0	—	10% in cholesterol	
	0·1 mg. P	4	0	—		
	0·01 mg. P	0	0	—	1% in cholesterol	
Deoxycortone acetate	2 mg. I	2	0	1·5	—	Active
	0·5 mg. I	0	0	Nil	—	
Testosterone	40 mg. I	0·5	3	28	—	Active
	40 mg. I	0	0·5	29	—	
	40 mg. I	0·5	0·5–1	—	—	
	21 mg. I	0·5	3	15·4	—	
	20 mg. I	0	1	10·2	—	
	20 mg. I	0	0	—	—	
	10 mg. I	0	0	3	—	
	10 mg. P	0·5	0·5	Some powder	—	
	5 mg. P	0·5	0	Some powder	—	
	5 mg. I	0	0	3	—	
	3 mg. I	0	0	Nil	—	
Methyltestosterone	2 mg. I	2	0	1	—	Active
	0·5 mg. I	1	0	—	—	
	0·3 mg. I	3	0	None	—	
	0·1 mg. P	0	0	—	—	

TABLE 3 (*cont.*)

Substance	Amount introduced in implanted segment	Response: Implanted segment	Response: Control segment	Residual weight of implant mg.	Remarks	Response when injected systemically
Testosterone propionate	20 mg. I	1·5	1·5	14·85	—	Active
	10 mg. P	0·5	0·5	Some powder left	—	
	3 mg. I	0	0	3	—	
17 Methyl dihydrotestosterone	10 mg. I	1–1·5	0	11	—	Active
	10 mg. P	0	0	Some powder left	—	
	1 mg. P	0·5	0	—	—	
Androsterone	16 mg. P	0	0	Some powder left	—	Inactive
	10 mg. P	0·5–1	0	—	—	
Androstenediol	20 mg. I	0	0	20·1	—	Not known
	10 mg. P	0	0	—	—	
Androstenediol acetate	3·5 mg. I	0	0	160	2% in cholesterol	Not known
Androstenedione	10 mg. I	0·5	0	7·8	—	Active
	10 mg. P	0·5	0	Some powder left	—	
Dehydroisoandrosterone	20 mg. I	1	1	17·6	—	Inactive
	10 mg. P	0	0	—	—	

I = pellet; P = powder.

A number of the findings call for discussion. Ethisterone was found to be effective in doses of 1 and 2 μg. It is, therefore, very nearly, if not quite as effective as progesterone. On systemic administration this substance is, however, decidedly less effective than progesterone. An explanation of this may be found in the fact that, as shown by Emmens (1941), ethisterone is a pro-oestrogen. Hence, on systemic administration, some of the substance undergoes metabolic changes and becomes active as an oestrogen. The progestational response to systemically injected ethisterone, therefore, will depend on the amount of the substance which reaches the endometrium unchanged.

Pregnenolone and deoxycortone acetate are active on local as well as systemic administration. Testosterone shows an interesting discrepancy between its local and systemic activity. The table shows that even in large doses (40 mg.) testosterone has no local progestational effect. On the other hand, Klein & Parkes (1937) obtained an effect on systemic injection of 20 mg., while Robson (1937) obtained a response with 12 mg. of testosterone propionate. The most probable explanation would appear to be that testosterone undergoes chemical change in the body into a substance which has a direct progestational effect. In agreement with this hypothesis is the finding, clearly shown in Table 3, that with larger doses of testosterone a progestational response may be produced in the unimplanted control segment. This is

explicable as the effect of testosterone which has undergone chemical change after being absorbed into the circulation. The table shows that methyl testo-

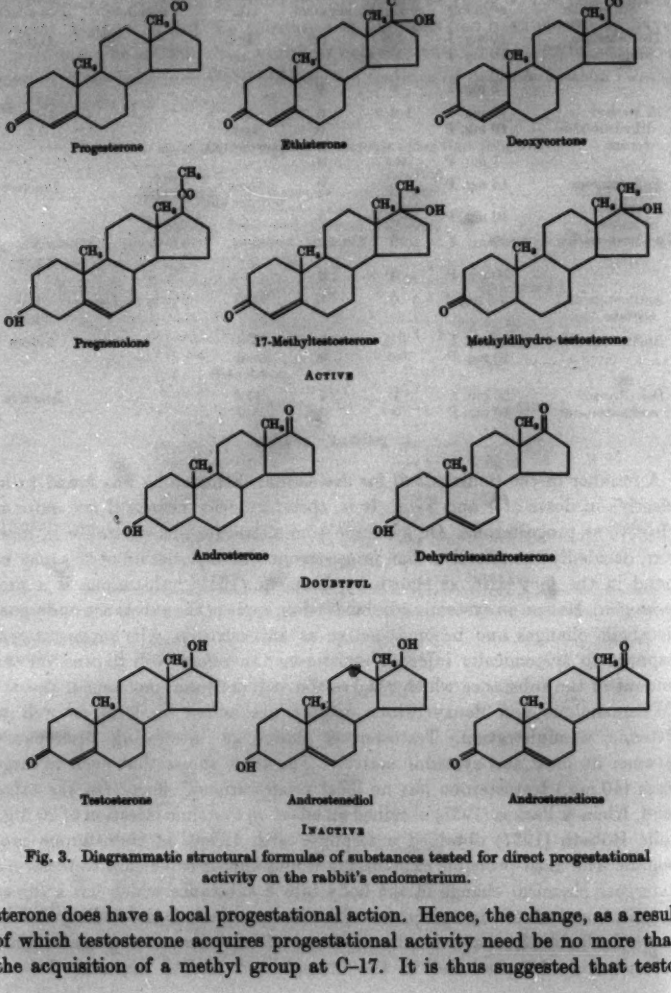

Fig. 3. Diagrammatic structural formulae of substances tested for direct progestational activity on the rabbit's endometrium.

sterone does have a local progestational action. Hence, the change, as a result of which testosterone acquires progestational activity need be no more than the acquisition of a methyl group at C–17. It is thus suggested that testo-

sterone, as such, is without progestational activity, but undergoes chemical change in the body to acquire such activity and it may be termed a pro-progestogen by analogy with the pro-oestrogens described by Emmens.

Of the remaining compounds tested only 17-methyldihydrotestosterone showed definite local activity. The effect of testosterone propionate and dehydroisoandrosterone and androsterone must be regarded as doubtful since changes in the control and implanted segment were equal, or, as in the case of the last-mentioned substance, only a borderline response was obtained. The fact that some of these substances are active on systemic administration is probably due to the fact that they also are pro-progestogens. This also applies to androstenedione which appears to be ineffective locally.

Consideration of the structural formulae of the steroids tested (see Fig. 3) suggests that there are certain relationships between chemical structure and direct progestational activity. Ethisterone, pregnenolone and deoxycortone, like progesterone, have a side chain of two carbons at C-17 and are all active. As shown by the case of pregnenolone, the ketone group at C–3 does not appear to be essential; this also applies to the existence of the double bond in the nucleus. Methyl testosterone and methyldihydrotestosterone, with only one carbon atom at C–17, also have local activity, while all the other compounds tested, which lack this feature also lack activity. The essential criteria for direct progestational activity, therefore, appear to be a steroid nucleus with a side chain of at least one carbon atom at C-17. Since Selye & Masson (1943) point out that no compounds with a side chain of more than four carbon atoms have been found to be active, the essential feature can be more clearly defined as a side chain of at least one but not more than four carbon atoms at C-17. We have no information as to the specificity of this side chain. The presence of a double bond in the nucleus is inessential. The position of an existing double bond appears to be, in the range of compounds studied, immaterial.

Inhibition of systemic progesterone by various steroids given locally

Some experiments already described showed that testosterone may produce less progestational proliferation at the site of implantation than in the rest of the uterus. This suggested the possibility that testosterone may produce an inhibition of the progestational reaction localized to the site of implantation, and experiments were therefore performed to test this possibility. Rabbits prepared in the usual way were injected systemically with progesterone and implanted locally with testosterone or certain other substances. Control experiments showed that the mere ligation of a part of a horn did not influence the reaction to progesterone given systemically. Implantation of cholesterol powder (10 mg.) produced no inhibition. The results are shown in Table 4. It will be seen that testosterone, dehydroisoandrosterone and androstenediol

were effective, androsterone was rather less effective and androstenedione produced no significant effect in a dose of 10 mg.

TABLE 4. Antagonism between progesterone given systemically and various steroids implanted into the uterine horn

(All animals received 1 mg. of progesterone by subcutaneous injection.)

Animal no.	Steroid implanted locally	Dose (mg.)	Progestational response in implanted segment	Control segment	Cholesterol (10 mg.) control segment
158	Testosterone	20	0·5	4	—
182	Testosterone	3	0	3	—
216	Testosterone	1	0·5	4	—
234	Testosterone	1	0	3	—
249	Testosterone	1	2	3	—
253	Testosterone	10	0·5	3	3
263	Testosterone	10	0·5	3·5	4
215	Dehydroisoandrosterone	10	0·5	4	—
235	Dehydroisoandrosterone	1	0·5–1	3	—
247	Dehydroisoandrosterone	1	1	3	2·5
251	Dehydroisoandrosterone	1	0	3·5	—
270	Dehydroisoandrosterone	10	0	3	3
238	Androsterone	1	3	4	—
264	Androsterone	10	0·5–1	3	3·5
230	Androstenedione	10	3	4	—
269	Androstenediol	10	0	3	3

An inhibitory effect was also demonstrated when testosterone was implanted together with progesterone. Thus, in one animal, 50 μg. of progesterone (as a 5% mixture in cholesterol) implanted locally produced a reaction of 4, whereas this same dose, implanted at another site together with 20 mg. of testosterone, produced no definite progestational proliferation (0·5). In another animal, 20 μg. of progesterone (as a 1 % mixture in cholesterol) gave a response of 2·5, but the same amount of progesterone implanted with 40 mg. of testosterone at one site, and with 3 mg. of testosterone at another site, produced no progestational proliferation in either of these sites.

SUMMARY

Solid implants of progesterone, or progesterone in cholesterol, and certain other steroids placed in the rabbit's uterus produce a progestational effect confined to the endometrium of the implanted segment, provided that the dose is not large enough to produce a systemic effect. 1% progesterone in cholesterol administered in this way to ovariectomized, oestrogen-primed adult rabbits gives reasonably uniform responses with a median effective dose of 1·08 μg. progesterone. This response is suggested as the basis of a method of assay for small doses of progesterone. When, after ovariectomy, the oestrogen administration is omitted the minimal effective dose rises as the period of oestrogen deprivation is lengthened. Ethisterone, deoxycortone acetate, pregnenolone, 17-methyltestosterone and 17-methyldihydrotestosterone also possess local, direct progestational activity. Ethisterone is almost as effective as pro-

gesterone in this respect. The essential factor in the chemical constitution of steroids having direct, local progestational activity appears to be the possession of a side chain of at least one carbon atom at C-17. Testosterone and possibly other steroids, though lacking direct progestational activity, appear to acquire it after chemical change within the body and may be termed pro-progestogens.

Testosterone and some other steroids, implanted into the uterus, can suppress the effects of progesterone and other steroids with progestational activity implanted in the same segment or given by systemic injection.

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REFERENCES

Emmens, C. W. (1941). *J. Endocrinol.* **2**, 444.
Finney, D. J. (1947). *Probit Analysis.* Cambridge University Press.
Hisaw, F. L., Greep, R. O. & Fevold, H. L. (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 840.
Klein, M. & Parkes, A. S. (1937). *Proc. Roy. Soc.* B, **121**, 574.
McGinty, D. A., Anderson, L. P. & McCullough, H. B. (1939). *Endocrinology*, **24**, 829.
McPhail, M. K. (1935). *J. Physiol.* **83**, 145.
Robson, J. M. (1937). *Quart. J. exp. Physiol.* **26**, 355.
Selye, H. (1940). *Proc. Soc. exp. Biol., N.Y.*, **43**, 343.
Selye, H. & Masson, G. (1943). *J. Pharmacol.* **77**, 301.
Shimkin M. B. & White, J. (1941). *Endocrinology*, **29**, 1021.

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THE EFFECTS OF ADRENALINE AND OF SYMPATHETIC STIMULATION ON THE DEMARCATION POTENTIAL OF MAMMALIAN SKELETAL MUSCLE

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The increase in twitch tension which follows a brief tetanus of a muscle has been shown to be associated with a diminution of the demarcation potential of the muscle. A similar increase in twitch tension and a similar reduction in demarcation potential can be produced by arterial injection into a muscle of suitable amounts of potassium chloride (Feng, 1938; Brown & Euler, 1938). The effects were so closely similar indeed, that they suggested that the potentiating action of a tetanus on twitch tension was due to a mobilization of K ions in the muscle.

The effect of adrenaline in increasing the twitch tension of a muscle has been shown to be due to a direct action upon the contractile mechanism of the muscle fibre (Brown, Bülbring & Burns, 1948), and recently Goffart & Brown (1947) have shown that the effectiveness of adrenaline on muscle is inversely proportional to the amount of potassium in the fluid bathing an isolated preparation. They pointed out also that fatigue, as such, is not a necessary condition for a clear potentiating action of adrenaline upon the muscle twitch. There are many superficial similarities between the effect of adrenaline and the post-tetanic potentiation: they are both effects upon the muscle fibre, their time courses are similar and they both appear to be associated with changes in the K^+ of the muscle. It appeared probable therefore, that a study of the effect of adrenaline upon the demarcation potential would be profitable. Adrenaline has been found to produce small but constant changes in it, but in the direction of increase of potential and not, as we had expected, in depolarization.

METHODS

The majority of the experiments were made on cats anaesthetized with chloralose, 0·6 mg./kg.; a few cats were decerebrated under ether. The tibialis muscle was prepared for arterial injection (Brown, 1938) and its nerve supply was severed. For tension recording the limb was held in a myographic stand, sometimes vertically, sometimes horizontally, and the tendon was connected to a flat spring myograph. The electrical records were taken with the limb horizontal and the muscle lying in medicinal liquid paraffin in a bath formed by the skin flaps. We cut the sciatic nerve on one

side in two cats, and in two we removed the abdominal sympathetic chains from the crura of the diaphragm to the 2nd sacral ganglia; the operations were made under nembutal anaesthesia and with aseptic precautions.

The demarcation potential was measured between a burned area at the tendinous end of the muscle and the belly of the muscle 2–3 cm. proximal to it. For electrodes were used wicks soaked in 0·9% sodium chloride solution, or 1% agar in a similar salt solution, in contact with chloride-coated silver wires. The electrodes were connected to the grids of a pair of cathode followers giving an effective input impedence of 90 MΩ. The output of the valves was taken to a moving coil galvanometer suitably damped. In our earlier experiments we read (at ¼ min. intervals) the deflexion of the galvanometer on a mm. scale; in the later experiments we recorded the deflexion on bromide paper on a slowly moving drum camera. The normal procedure was to place the recording electrodes close together on the belly of the muscle and to balance any observed p.d. to zero. One electrode was then placed on the burnt region of muscle, and the absolute value of the demarcation potential was measured or recorded at low sensitivity (1–10 mm./mV.). The sensitivity was then increased to 1–10 cm./mV. and the circuit was balanced so that the spot was in the centre of the scale. During each experiment frequent checks were made of the interelectrode potential and of the absolute value of the p.d.

We found it desirable to make an annular burn and to wait about ½ hr. afterwards before beginning recording. Even then drift towards a diminished p.d. often occurred and was a nuisance. Recording was made much easier by immersion of the muscle in paraffin; the wick electrodes made entirely satisfactory contact with the muscle under the paraffin, even when they were frequently moved. The cat and the paraffin bath were kept between 37° and 39° by a source of radiant heat.

L-Adrenaline base (B.D.H.) was dissolved with HCl in saline just before use and diluted to give the appropriate concentration for close arterial injection in a volume of 0·25 c.c. DL-Noradrenaline hydrochloride (I.G. Farbenindustrie) and DL-isopropyl-noradrenaline sulphate (B.W. and Co.) were dissolved in saline and similarly diluted.

RESULTS

Effect of adrenaline on twitch tension

Since relatively little is known about the effects of adrenaline on unfatigued mammalian muscle, we made a preliminary study of the effects of arterial and intravenous injection of adrenaline on the tibialis anterior muscle.

The first administration in an experiment of 0·5–20 μg. adrenaline in 0·25 c.c. saline invariably produced an increase in the tension evoked by single maximal motor nerve volleys at 10 sec. intervals. The increase in tension varied between 3 and 15%, but occurred regularly without the necessity of fatiguing the muscle. Subsequent injections, made within an hour of each other, were progressively less effective, the 3rd and 4th generally failing to have any noticeable action. The onset of the increase in tension is gradual, the peak of the potentiation being reached in 2–5 min. Thence the tension declines until the potentiation is over in 15–20 min. (Fig. 1). Thereafter there may be some decline in tension, but it is difficult to distinguish this from the normal decay of the preparation. With these doses, and with this frequency of stimulation, we did not observe the immediate depression of the twitch such as is described by Luco (1939) and by Bülbring & Burn (1939), under rather different experimental conditions.

The time course of the potentiation is not related to that of the general vascular effects of the adrenaline, as judged by the changes in arterial blood

pressure. The peak of the rise in blood pressure is attained before the peak tension, and the blood pressure has returned to its previous level before the

Fig. 1. Cat, 3·7 kg. chloralose. Contraction of tibialis anterior in response to single maximal nerve volleys at 6/min. At arrow, close arterial injection of 5 μg. adrenaline. (The record was made with a weighted recording lever to produce an 'overthrow'.)

Fig. 2. Cat, 2·4 kg. chloralose. *a* and *c*, arterial blood pressure; *b* and *d*, contractions (downwards) of tibialis with single maximal nerve volleys at 6/min. At arrows, arterial injection of 5 μg. adrenaline. Between *b* and *c* intravenous injection of 7·5 mg. ergotamine tartrate. The blood pressure and muscle records were taken simultaneously, but at different speeds of recording surface.

potentiation is maximal. Abolition or reversal of the blood pressure changes by the administration of ergotoxine, ergotamine or dibenamine does not significantly alter the effect of the adrenaline on muscular tension (Fig. 2). Exact

comparison of the effects of adrenaline before and after ergotoxine or other substances, is made difficult by the tachyphylaxis which is such a characteristic feature of the action of adrenaline on muscle tension.

Effect of adrenaline on demarcation potential

The arterial injection of 10 μg. adrenaline into a muscle causes an increase in the demarcation potential which in degree and time course is very like the increase in tension produced by a similar injection. Fig. 3 shows such an effect; in this experiment the demarcation potential was quite steady before the injection,

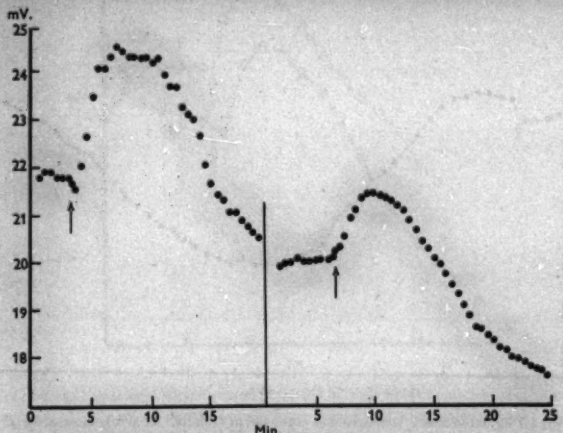

Fig. 3. Cat, 3 kg. nembutal. Demarcation potential of tibialis. Effect of close arterial injection of 10 μg. adrenaline at arrows. An interval of 40 min. elapsed between the two records.

but showed a decline after crossing its original base-line. This has been a constant phenomenon. We have, unfortunately, been unable accurately to correlate the two phases of the changes in polarization with the changes in twitch tension by recording the two phenomena simultaneously, since the electrodes are often dislodged by the movement of the stimulated muscle and observation of the demarcation potential, at the high sensitivity needed to detect the changes in polarization, can seldom be maintained for long enough to follow the whole course of the phenomenon.

It appears to us that there is a reasonably close correlation between the time to maximum polarization and the time to the peak of the increase in twitch tension. Thus the peak of the increase in potential was reached in 2–3 min. in a number of experiments, figures which correspond closely enough with those for the changes in twitch tension. It is difficult to measure the end of the effect

on tension, although we have generally assumed it to be over in about 15 min., a time which again corresponds with estimates of the end of the period of polarization; but exact determination of this point is made even more difficult by the onset of the decline of demarcation potential. We have been able, in a few experiments, to follow the whole course of the changes, and an example is given in Fig. 4. If allowance is made for probable direction of drift, the polarization appears to be over in this instance in 5 min. and the depolarization to have ceased in some 13 min., and this general ratio of duration has been evident in all

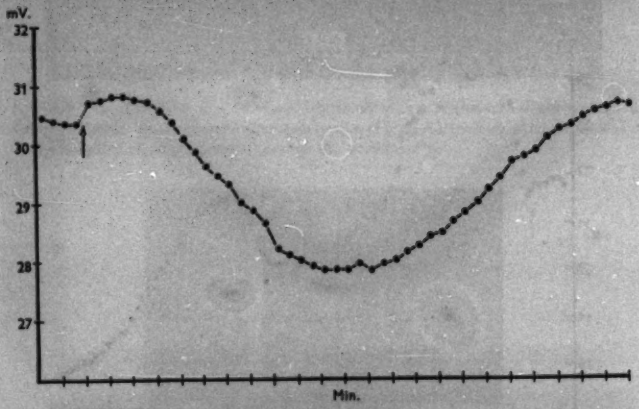

Fig. 4. Cat, 1·7 kg. chloralose. Demarcation potential of tibialis. At arrow close arterial injection of 10 μg. adrenaline.

the experiments in which the stability of the base-line has enabled us to assess it. We can, at all events, say that the polarization is followed by a depolarization of longer duration and greater extent.

We have no reason to believe that the polarization is an accidental effect, since it occurs with regularity after both arterial and intravenous injection of adrenaline. Mechanical disturbances of any sort are, of course, liable to upset the recording, but we have only occasionally observed changes in the direction of increased polarization after a control, saline injection, and these have always been in shape quite unlike those after adrenaline. We have similarly obtained negative controls with the injection of saline solutions at a variety of temperatures from room temperature to 40° C.

The electrical changes produced in muscle, like the mechanical changes, diminish with repeated doses of adrenaline. Thus, in a number of experiments, first doses have produced increases in demarcation potential of 5–10% and

second doses increases of 3–5%; third doses have usually been much less effective.

Denervated muscle. Adrenaline produces in muscle, after degeneration of its nerve supply, electrical changes indistinguishable from those seen in normal muscle. Fig. 5 shows the effect of 10 μg. of adrenaline in the normal leg and in a muscle 7 days after section of the sciatic nerve.

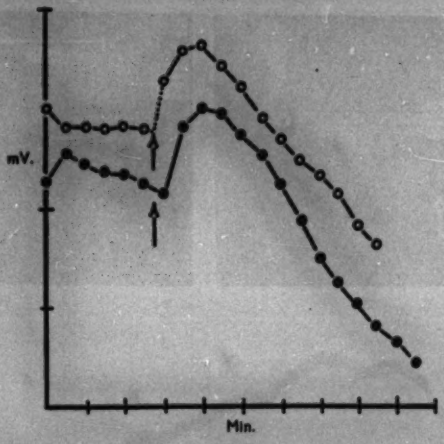

Fig. 5. Cat, 2·1 kg. chloralose. Demarcation potential of both tibialis muscles (absolute value not determined). Upper record normal muscle, lower record from muscle denervated 7 days before. At arrow, close arterial injection of 10 μg. adrenaline.

Neuromuscular block. The production of complete neuromuscular block by the arterial injection of some 200 μg. of D-tubocurarine chloride or of 20 μg. decamethonium iodide did not diminish the effectiveness of adrenaline in increasing the demarcation potential. After decamethonium indeed, which itself depolarizes muscle (Brown, Paton & Vianna Dias, 1949), the effect of adrenaline appears to be greater.

Effect of sympathectomy. In two cats we removed the abdominal sympathetic chains 2 weeks before examining the effects of adrenaline. Sympathectomy in no way alters the effect of adrenaline on demarcation potential.

Effect of other sympathomimetic amines

Both arterial and intravenous injection of DL-noradrenaline produce effects on tension which are generally comparable, but often less than those produced by adrenaline (cf. West & Zaimis, 1949). The effect on demarcation potential does not differ from that produced by adrenaline. Isopropyl-noradrenaline was

a substance of special interest to us, since its only known effects on smooth muscle are inhibitory; it causes the characteristic increase in twitch tension and resting potential. It differs from the other substances which we have used in producing, on occasion, much longer lasting polarization, and this again runs parallel with the changes in twitch tension which can, apparently, persist much longer than they do with other sympathomimetic substances.

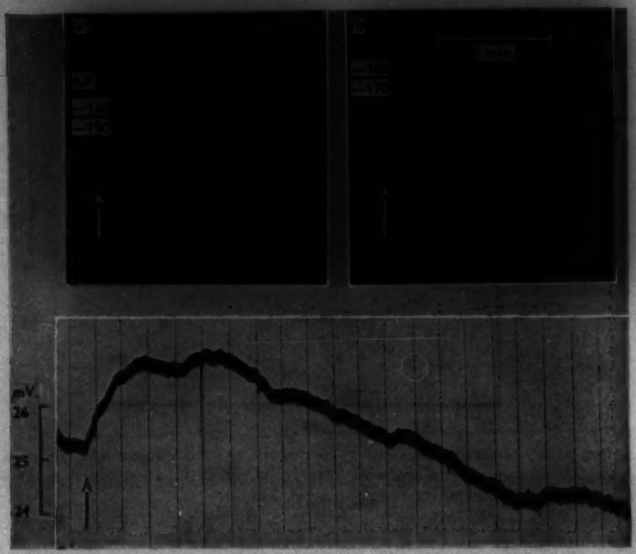

Fig. 6. Cat, 2·4 kg. chloralose. Effect of close arterial injection of 10 μg. adrenaline on blood pressure (*a*) before and (*b*) after administration of 7·0 mg. ergotamine. The lower record is of the demarcation potential of tibialis and corresponds to upper record (*b*). Time marks, 1 min.

Effect of antagonistics to adrenaline

Dibenamine, ergotoxine and ergotamine, which reverse the vaso-pressor action of adrenaline leave unchanged the effects of adrenaline on the demarcation potential (Fig. 6), just as they do not alter the effect on twitch tension (Fig. 2).

Effect of other substances

Brown & Euler have already illustrated the effect of KCl on twitch tension and demarcation potential, but we have included an illustration (Fig. 7) of the effect on demarcation potential of an injection of KCl taken under conditions identical with those of the adrenaline experiments. The initial, profound

depolarization is synchronous with the tetanic contraction which arterial KCl usually produces; the subsequent, lasting depolarization is associated with the increase of twitch tension.

Quinine. There is some similarity between the effects of quinine and of adrenaline on muscle, in that both appear to evoke an increase in twitch tension

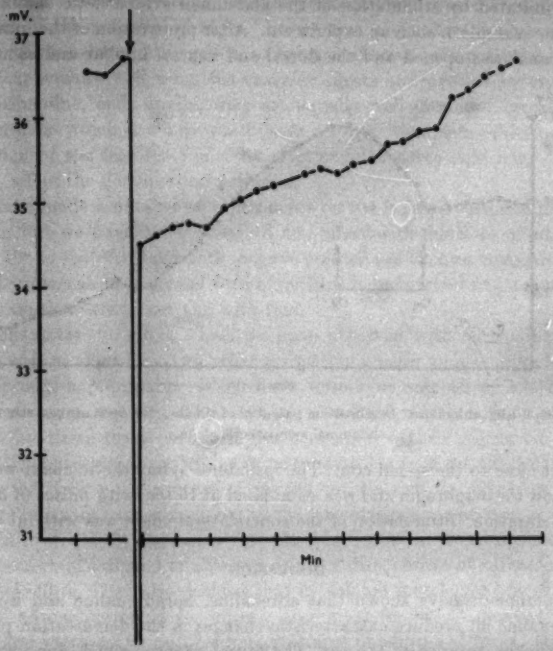

Fig. 7. Same experiment as Fig. 3. At arrow, close arterial injection of 2·5 mg. KCl.

by a slowing of the propagation of the excitation wave (cf. Harvey, 1939; Brown *et al.*, 1948). We thought it desirable, therefore, to investigate again the effect of quinine on demarcation potential. We were able to confirm Harvey's finding that quinine given arterially in doses of 1 mg. is without effect on the resting potential of the muscle.

Pituitary (posterior lobe) extract. Pituitary extract can produce an increase in the twitch tension of a fatigued muscle (Bülbring & Burn, 1940), and it causes vascular changes in a muscle which might be like those produced by adrenaline.

It has, however, no effect on demarcation potential when injected arterially in a dose of 1 i.u. diluted in 0·25 c.c. of saline. This solution so injected had a pH of 4·50, and it is interesting that such an acid solution itself had so little effect.

Effect of stimulation of sympathetic chain

The effects of the sympathomimetic amines on demarcation potential can be closely imitated by stimulation of the abdominal sympathetic chain. Fig. 8 shows an example of such an experiment. After preparation of the muscle, the spinal canal was opened and the dorsal and ventral lumbar and sacral roots

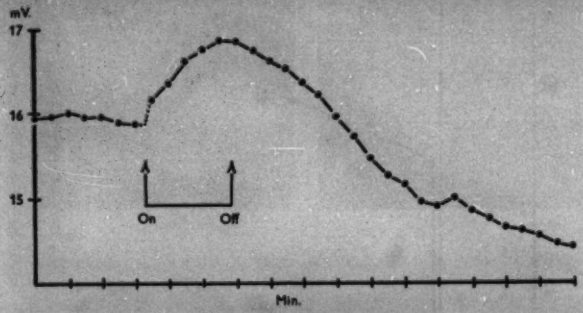

Fig. 8. Cat, 2·1 kg. chloralose. Demarcation potential of tibialis. Between arrows, stimulation of abdominal sympathetic chain.

were cut close to the spinal cord. The ipsilateral sympathetic chain was freed just below the diaphragm and was stimulated at 76/sec. with pulses of 3 V. and 1 msec. duration. Stimulation of the contralateral chain was without effect.

DISCUSSION

Our experiments have shown that adrenaline, noradrenaline and isopropylnoradrenaline all produce characteristic changes in the demarcation potential of the tibialis anterior of the cat. The initial change, which appears to run parallel with the increase in twitch tension of the excited muscle, is in the direction of an increase of the potential. We had expected, by analogy with the effects of injected KCl, that any change we might detect would be in the opposite direction. After adrenaline a depolarization does, in fact, occur, but at a time when the tension is declining after having reached its peak value. Feng and Brown & Euler ascribed the changes which occur in the post-tetanic potentiation to changes in the distribution of K ions because they were able to imitate both the post-tetanic increase in tension and the post-tetanic fall in demarcation potential by the arterial injection of KCl solutions. There is reason to believe, as we have pointed out earlier, that the effect of adrenaline is also associated

with movements of K ions, but our present experiments suggest that the changes produced by adrenaline are more complex than the increased exchange and loss of K^+ which occurs when a muscle is tetanized. Experiments now in progress by one of us (M.G.) and Dr W. L. M. Perry, using labelled K^+, suggest that there is a diminished exchange of K^+ during the polarization with adrenaline and an increase in K^+ exchange during the depolarization.

We have much evidence to exclude the participation of vascular effects in the causation of the potential changes. In the first place there is no correlation between the vasoconstriction produced by adrenaline and the potential changes, they occur equally well when the vascular effects are reversed by ergotoxine and dibenamine, and, finally, they are equally well produced by isopropyl-noradrenaline which has only vasodilator actions. Complete occlusion of the circulation of the legs for 3 min. by clamping the aorta does not, strangely enough, affect the demarcation potential.

We have made a number of experiments on the isolated sartorius muscle of the frog, but we have never observed any effect with dilutions of adrenaline from $1:10^8$ to $1:10^{12}$. Many authors have pointed out that no augmentation of twitch tension can be observed with adrenaline in unfatigued frog's muscle and our observations are in keeping with this.

The characteristic effect which we have observed with adrenaline can be equally well produced by two other sympathomimetic amines, noradrenaline and isopropyl-noradrenaline which have actions on smooth muscle differing widely in degree and sign. Their action on striated muscle is, moreover, unaffected by those drugs which suppress or reverse their effects on smooth muscle. There seems, therefore, to be no relation between the effect of adrenaline on the contractile process of striped muscle and its effect on specific receptors in smooth muscle related to the terminations of nerves.

Brown *et al.* (1948) concluded that the augmentation by adrenaline of the twitch tension of fatigued muscle was due to a direct effect of adrenaline upon the muscle fibre. They also decided that in isolated muscle the 'decurarizing' effect of adrenaline could be entirely accounted for by an increase in fibre tension and was not attributable to recruitment of additional fibres. Maddock, Rankin & Youmans (1948) have shown that a dose of adrenaline given intravenously in the dog can reduce a partial curarization of the tibialis muscle, and that this effect is abolished by dibenamine; we have been able to confirm this in the cat. It appears, therefore, that if adrenaline has, in fact, an effect upon neuromuscular transmission, it is different from its direct effect on the muscle fibre in its sensitivity to dibenamine. Dibenamine has been shown to diminish the liberation of K^+ from the liver by adrenaline (West, 1949), and this gives further support to the suggestion made by Brown *et al.* that some of the effects of adrenaline in the whole animal might be ascribed to the liberation of K^+ from tissues other than the muscle.

The amounts of adrenaline which we have used have been greatly in excess of any likely to be present in the blood stream during normal life. But the fact that the effect on demarcation potential can be reproduced by stimulation of the sympathetic supply to the lower limbs suggests that similar changes can occur in normal muscle.

Our experiments have given us no clue as to the nature of the fundamental change underlying the changes in the potential. We know that adrenaline can cause an increase in twitch tension of the individual fibre and that this is accompanied by, or due to, a retardation in the progression of the excitation wave; it is perhaps not surprising that such changes should be accompanied by changes in the resting potential of the muscle membranes.

SUMMARY

1. Close arterial injection of 0·5–20 μg. of adrenaline into the tibialis anterior muscle of the cat causes an increase of 2–15% in the tension response to single maximal nerve volleys. The effect is independent of muscular fatigue.

2. A similar arterial or intravenous injection of adrenaline causes an increase up to 10% in the demarcation potential of the muscle which follows approximately the increase in twitch tension.

3. The increase in demarcation potential is followed by a depolarization of greater magnitude and longer duration.

4. These effects are produced also by DL-noradrenaline and by DL-isopropylnoradrenaline, and they are unaffected by doses of ergotoxine or dibenamine sufficient to reverse the vasopressor actions.

5. These effects of sympathomimetic amines can be observed in denervated muscle and in muscle in which complete neuromuscular block has been produced by D-tubocurarine chloride or by decamethonium iodide.

6. Stimulation of the abdominal sympathetic chain produces similar changes in the demarcation potential.

REFERENCES

Brown, G. L. (1938). *J. Physiol.* **92**, 22 *P*.
Brown, G. L. & Euler, U. S. v. (1938). *J. Physiol.* **93**, 39.
Brown, G. L., Bülbring, E. & Burns, B. D. (1948). *J. Physiol.* **107**, 11*S*.
Brown, G. L., Paton, W. D. M. & Vianna Dias, M. U. (1949). *J. Physiol.* **109**, 15*P*.
Bülbring, E. & Burn, J. H. (1939). *J. Physiol.* **95**, 203.
Bülbring, E. & Burn, J. H. (1940). *J. Pharmacol.* **68**, 150.
Feng, T. P. (1938). *Chin. J. Physiol.* **13**, 79.
Goffart, M. & Brown, G. L. (1947). *C.R. Soc. Biol., Paris.*, **141**, 958.
Harvey, A. M. (1939). *J. Physiol.* **95**, 45.
Luco, J. V. (1939). *Amer. J. Physiol.* **125**, 196.
Maddock, W. O., Rankin, V. M. & Youmans, W. B. (1948). *Proc. Soc. exp. Biol., N.Y.*, **67**, 151.
West, G. B. (1949). Personal communication.
West, G. B. & Zaimis, Eleanore, M. (1949). *J. Physiol.* (in the Press).

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SENSITIZATION OF SENSORY RECEPTORS IN THE FROG'S SKIN

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Papers on the innervation of the frog's skin have usually described two main groups of sensory fibres, the fast and the slow, with high and low action potentials, conduction velocities, and adaptation rates respectively. Fessard & Segers (1943) have now described a third group, called by them A_2 fibres, which have properties intermediate between the other two; their classification has been adopted in this paper. Thus when a weight is dropped on to the skin, the resulting nervous discharge consists of three parts: a short rapid burst of impulses in A_1 and A_2 fibres, followed by a continued discharge of A_2 impulses, which may last as long as the weight remains on the skin. If the weight is sufficiently heavy, there is a slowly rising and falling discharge of slow impulses, which may persist long after the stimulus has been removed. Hogg (1935) suggested that this behaviour of the slow fibres pointed to the release of some substance from the injured tissues, which could act either as a direct or additional excitant to the sensory receptors. This view was supported by Echlin & Propper (1937), who showed that scraping the skin would cause 'sensitization' of the slow fibre endings: they defined sensitization as a condition of the skin in which a standard weight, when lowered on to a particular spot, caused an increased slow fibre discharge. Scraping had already been shown by Feng (1933) to cause a gradual inhibition of the fast fibre response by release of some substance, probably potassium, from the damaged cells. Sensitization was tentatively assigned a similar cause, though it was not found possible to demonstrate it convincingly with potassium.

The work described in this paper started as an attempt to investigate the phenomenon of sensitization further, or at least to confirm or exclude the part played by potassium. It was also tempting to compare it with the hyperalgesia described by Lewis after injury to human skin, and indeed the comparison is not so unwarranted as might be imagined, since an accompanying phenomenon, the triple response, was demonstrated in 1929 on the frog's tongue by Grant & Jones. They also showed the presence of a histamine-like substance in its

skin. One of the methods by which Lewis obtained hyperalgesia was by antidromic stimulation of cutaneous nerves, and this paper is mainly an account of similar experiments in the frog.

METHOD

Isolated dorsal skin-nerve preparations were used in most of these experiments. The skin was placed on a small rigid platform, which formed the top of a moist chamber containing the electrodes, and into which the nerve was threaded through a slit. The electrodes, both for stimulating and recording, were of the usual silver, silver chloride, Ringer, cotton wick, variety; recording was by means of a condenser coupled amplifier going to a Matthews' oscillograph for photographing the impulses, and a cathode-ray tube for viewing them. The electrical stimulator was a neon flashing circuit, with the lamp discharging through a potentiometer and transformer; the shock artefact was partially balanced out by negative feedback into the amplifier.

The skin was stimulated mechanically by means of a lever, which could be loaded with various weights and lowered on to it. The movement of the lever was controlled by a dashpot, and its point consisted of a glass sphere of diameter 3 mm., so arranged that it could always be brought to bear on the same region of skin. In some experiments where a vibratory stimulus was used, a similar glass sphere was connected by a rigid rod to the armature of a loudspeaker, which was driven off the a.c. mains at 50/sec. The amplitude of the vibration was controlled by a potentiometer.

Each preparation was soaked in Ringer solution for about 10 min. before the experiment began, otherwise the excitability rapidly declined. They were also irrigated continuously with Ringer during the experiment.

RESULTS

Part I

In confirming the results of Echlin & Propper, it has been found that sensitization is not confined to the slow fibres alone, but also affects the A_2 group. Fig. 1, which shows the nervous response to a standard weight before and after

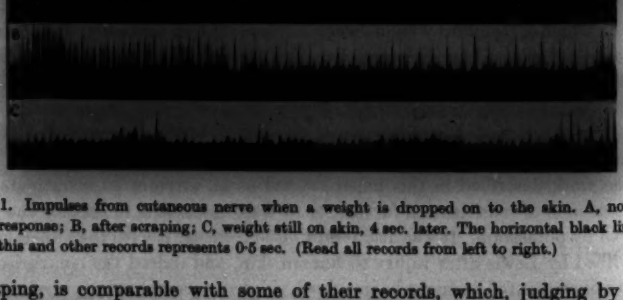

Fig. 1. Impulses from cutaneous nerve when a weight is dropped on to the skin. A, normal response; B, after scraping; C, weight still on skin, 4 sec. later. The horizontal black line in this and other records represents 0·5 sec. (Read all records from left to right.)

scraping, is comparable with some of their records, which, judging by the width and height of some of their 'slow' impulses relative to fast ones in the same preparation, show the same thing, though they classified the impulses

differently. In all these confirmatory experiments there seemed to be no relation between the spread of sensitization in the slower fibres, and of inhibition in the faster ones. The latter effect is almost certainly caused by release of potassium (Feng, 1933), and both it and sensitization are destroyed by irrigation with Ringer solution. A critical experiment was therefore to irrigate a sensitized skin with Ringer solution containing 0·2% KCl, a concentration insufficient to cause immediate inhibition of the fast response, and yet one which is not likely to be exceeded in the fluid from injured tissues. This solution destroyed sensitization as rapidly as ordinary Ringer solution. Thus potassium seems to be excluded as a sensitizing agent, and Echlin & Propper's effect must be a separate phenomenon.

Antidromic stimulation of cutaneous nerves

In a preliminary series of experiments the two recording electrodes were placed on the peripheral end of a nerve in the isolated skin preparation, and the stimulating electrodes on the cut central end; the lever was then lowered several times on to a particular spot on the skin, until a standard response was obtained and the value of the weight was so adjusted that the A_2 or slow discharge consisted of only one or two impulses. The nerve was then stimulated antidromically at 50/sec. for about 5 sec., after which the weight was lowered and the impulses counted again. In five out of seven such experiments there was an increased discharge, and often the endings would fire off spontaneously either during or after the antidromic stimulation. For example, in one preparation there was no A_2 discharge when the weight was lowered before stimulation, and eleven impulses after it. Sometimes this sensitization would last only for a few minutes, and on other occasions for an hour or more, and preparations have been found in which the endings continued to fire off spontaneously for over a quarter of an hour. As washing had no effect, it appears that any chemical changes taking place must be within the skin.

In a few cases the vibrating rod was used as a test mechanical stimulus, and similar sensitization could be demonstrated. For example, in one preparation a slow fibre fired off to about one in every ten of the vibratory stimuli, but after antidromic stimulation, responded to every alternate one, i.e. at 25/sec.

These experiments suffered from two main disadvantages; first there was no indication whether the effect was a direct one on the end-organs, or was due to the release of some substance, and secondly, the strong shocks which had to be used had a damaging effect on the nerve itself. Other preparations were therefore designed in an attempt to avoid them.

Double skin preparation. If antidromic stimulation really causes release of some substance at the nerve endings, it ought to be possible to sensitize another preparation in contact with the first. This was tested using the double skin preparation. It consisted of two pieces of skin with their undersides in contact,

and with one nerve from each threaded through the opposite skin (Fig. 2). This arrangement was chosen because the inner surface of the skin is so much more permeable to drugs than the outside. Care was taken to see that the regions supplied by both nerves were more or less coincident.

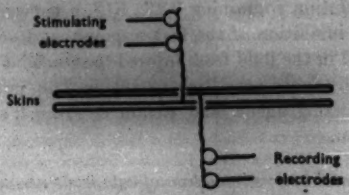

Fig. 2. Double skin preparation.

In several such preparations definite sensitization was found in one skin, after stimulation of the nerve going to the other. Table 1 shows the slow fibre discharge when the weight was dropped in two of these experiments. Sometimes a slight spontaneous discharge was observed during stimulation, and this was even seen in preparations in which no sensitization could be demonstrated; a possible explanation is that the part of the skin which was tested by the weight, did not happen to lie next to the region from which the sensitizing agent was being liberated.

Table 1. Double skin preparation. Each figure represents the number of slow impulses discharged on a separate lowering of the weight

Preparation	Before stimulation	After stimulation
1	0, 0	26, 5
2	5, 6, 5	12, 26, 11, 6, 25, 11, 20, 5

Double nerve preparation. There is very extensive overlap between skin areas innervated by adjacent nerves. Woollard *et al.* (1940) have shown that in human skin the small fibres form a plexus, though there is no actual continuity between branches from different axons. Similar plexuses are found in frog's skin, and it is possible that anything liberated from one fibre, would affect others in the neighbourhood. Experiments were therefore done, stimulating one cutaneous nerve electrically, and recording from the one next to it. This arrangement was known as the 'double nerve preparation' (Fig. 3).

In fifteen out of twenty-three preparations, sensitization was found in the region of skin supplied by both nerves. The electrodes were so arranged that the recording leads could first be placed on one nerve, and then on the other, without disturbing their position. Hence it was easy to find the area of overlap, and the weight was used as a test stimulus there. The results of some experiments

are set out in Table 2, where the standard weight was applied before and after stimulation of the nerve. Sometimes the weight was dropped at regular intervals of a minute, and the results are plotted in Figs. 4 and 5. In Fig. 5 it

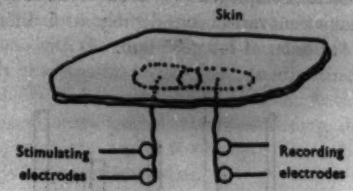

Fig. 3. Double nerve preparation.

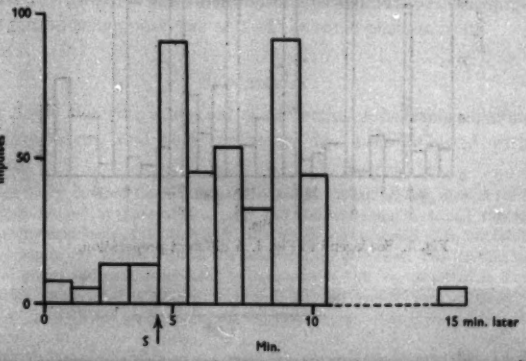

Fig. 4. Double nerve preparation. The height of each block represents the number of A_α impulses discharged after a separate dropping of the weight. At S one of the nerves was stimulated antidromically for 5 sec.

TABLE 2. Double nerve preparation. Each figure represents the number of A_α or slow impulses discharged on a separate lowering of the weight

Exp.	Before stimulation	After stimulation
1	12	40
2	0, 0, 0	37, 52, 26
3	0, 1, 1, 0, 0	3, 14, 3, 19, 0, 3, 12, 2
4	0, 3, 0, 1, 1	24, 30, 23
5	8	23
6	0	33
7	6	74

can be seen that sensitization disappears very rapidly, but this was exceptional. Since stimulation with the weight must have a direct and profound action on the endings themselves, these results are not to be taken quantitatively, but they do provide undeniable evidence of a raised excitability.

It was not found possible to differentiate degrees of sensitization with electrical stimulation of different durations and frequencies, since the effect was not sufficiently repeatable to allow such measurements to have any meaning, but the usual ones employed were about 5 sec. at 50/sec. The pressures used for tactile stimulation varied considerably with different preparations, but were usually of the order of 1–2 g./sq.mm.

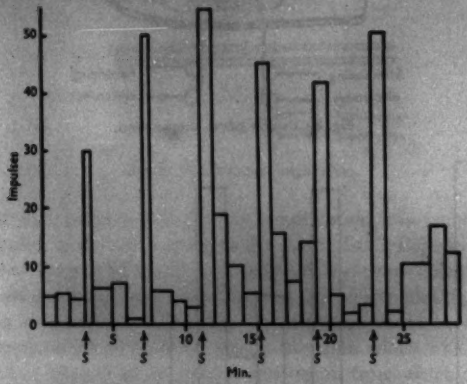

Fig. 5. See legend to Fig. 4. A different preparation.

Fig. 6. Double nerve preparation. Discharge when weight is dropped. A, normal response; B, after antidromic stimulation; C, weight still on skin, continuous with B.

Vascular changes. In mammals, hyperalgesia is usually associated with redness of the skin, and antidromic stimulation of sensory nerves is known to cause vasodilation. It therefore seemed desirable to find whether analogous effects could be demonstrated in the frog. Grant & Jones (1929) found that the peripheral flare could still be caused by injury immediately after section of the

glossopharyngeal (sensory) and hypoglossal (motor) nerves, but not after degeneration; the local responses remained unchanged. They also found that after occlusion of the blood supply to the tongue, the flare developed after injury immediately the circulation was released, showing that it must have been caused by something already present, i.e. a chemical agent. All their experiments were done with the tongue kept in the mouth, and not pinned out; the frogs were lightly anaesthetized with ether. In repeating their experiments, the occurrence of the triple response was confirmed. Antidromic stimulation of the glossopharyngeal nerve caused intense reddening, developing in under a minute, on the appropriate side of the tongue; the red area corresponded exactly with that giving a tactile response.

The vascular changes could not be correlated with any sensitization, since back stimulation caused a large and continuous spontaneous discharge, which there is no reason to suppose was part of the same phenomenon.

Skin extracts

Grant & Jones describe a method of extracting a histamine-like substance from the frog's skin, and their procedure has been followed with a few modifications.

12 g. of fresh finely chopped frog's skin were soaked in 150 ml. of 98 % alcohol for 48 hr. The mixture was then filtered, evaporated to dryness, and dissolved again in alcohol; this was done in an attempt to eliminate some of the inorganic salts, which were assumed to be less soluble in pure alcohol, than when it was diluted with all the fluid from the skin itself. The solution was divided into two equal parts, and each evaporated to dryness; one half was dissolved in 2 ml. Ringer, shaken with ether to remove the fat, and separated; this was known as the 'extract'. The other half was heated until all the organic matter had charred, but not long enough to volatilize the inorganic chlorides; the residue was dissolved in 2 ml. Ringer, and filtered; it was known as the 'fused extract', and was assumed to contain only the inorganic fraction of the extract.

The results obtained with these solutions were rather variable. On the tongue, the extract gave reddening and a spontaneous discharge, though none was obtained with KCl or fused extract. On the skin, especially when placed

TABLE 3. The figures show the number of impulses discharged per application of a standard weight

Solution on skin	No. of impulses
Ringer	0, 1, 1
0·2 % KCl in Ringer	0, 2, 5, 3, 5
Ringer	11, 4, 8, 19, 4, 2
Extract	56, 0 (Enormous spontaneous discharge)
Ringer	0 (Preparation practically inexcitable)

on the underside, there was usually a spontaneous discharge and sensitization with the extract, though often the endings ceased to respond after it had been on for several minutes. Typical results are given in Table 3. The figures under

the heading 'extract' were obtained after the spontaneous discharge had died down. A few minutes later the preparation became practically inexcitable.

The fused extract usually had no effect, but occasionally caused a slight spontaneous discharge, followed by a complete and rapid loss of excitability; it never caused prolonged sensitization. A very strong solution of KCl behaved similarly. No attempt has been made to prepare any less crude extracts, and some of the effects described are undoubtedly due to potassium, but this cannot account for the sensitization which has been observed with extract, unaccompanied by inhibition of the fast fibres. These prolonged effects are tentatively assigned to the action of some specific sensitizing agent.

Histamine (1 in 1000), when dropped on the skin, sometimes caused an enormous spontaneous discharge of fast and slow impulses, though Grant & Jones found no action with it on the tongue, nor on the blood pressure after intravenous injection, even in concentrations up to 1 %. In other preparations it had no effect at all, though the skin extract would cause a slight discharge.

Part II

Spontaneous discharges

Mention has already been made of the spontaneous discharges, which appeared during antidromic stimulation. They have been especially studied in the double nerve preparation, which was used for all the experiments described below, but have been observed in the other types as well. In forty preparations they have been found thirty-four times.

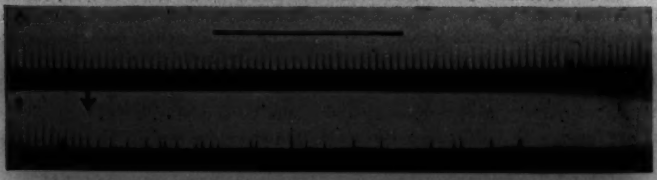

Fig. 7. Random spontaneous discharge in double nerve preparation. The large regular spikes are the shock artefact at 50/sec. The discharge can be seen beginning about two-thirds of the way along the top record. A, starts about 0·5 sec. after the beginning of stimulation, and B is continuous with it. Stimulation was stopped at the arrow; note the long after-discharge.

The discharges consisted of both fast and slow impulses, and were often completely random. They would either start immediately stimulation was begun, or build up gradually during a second or two, and sometimes would continue after it had stopped. Fig. 7 shows a slowly developing discharge of this kind. This was the usual response to stimulation at 50/sec. or more. At lower rates of stimulation, however (e.g. 5–20/sec.), after-discharges were rarely

seen, but it was found that often some of the spontaneous impulses would follow the antidromic impulses with a one-to-one relationship; in other words, one or more impulses would come up one nerve, going away from the skin, for every volley going down a neighbouring nerve towards it. Such impulses were known as 'following impulses'. They were not always easy to demonstrate, but the usual procedure was to tie off every dorsal cutaneous nerve, and then to try them in pairs, stimulating one and recording from a nearby one, until the phenomenon was found; in this way it was discovered in a very high proportion of preparations. The one-to-one relation was not invariable, but almost all grades of response were found, from a few impulses, or a random discharge, to as many as six different following impulses per shock (see Fig. 10). Sometimes these impulses would appear as soon as stimulation was started, and sometimes not until after a considerable latent period; in the latter cases, there seemed to be some relation between the latent period and the rate of stimulation. Figures from such an experiment are given in Table 4. These observations were made in a random order, with an interval of several minutes between each. They seem to suggest the building up of some excitable state dependent upon the number of shocks.

TABLE 4. Double nerve preparation. Experiment showing the dependence of the latent period before the beginning of the spontaneous discharge, on the rate of stimulation

Rate of stimulation	Latent period
12/sec.	7·5, —, 7·5, 6·9 sec.
24/sec.	3·0, 1·7, 2·4, 1·6 sec.

When a regular following impulse was found, its delay (i.e. the conduction time from one nerve to the other) might be anything between 3 and 100 msec., and on one occasion has been seen as long as 200 msec. In the majority of cases, particularly those with delays of over about 15 msec., it increased rapidly during stimulation, until the fibre began to respond only occasionally, and finally stopped altogether. After a short rest the whole sequence could be repeated exactly. Fig. 8 shows a following impulse of this kind, and in Fig. 9 the delay is plotted against the number of shocks for two different rates of stimulation; the lines end where the following impulse failed to appear at every shock, and it will be seen that this is at about the same delay for both; the impulse is described as becoming 'intermittent' here. Fig. 9 also shows how the increase in delay varies with the number of stimuli rather than their frequency, and indeed, on one occasion two similar graphs for two different frequencies could be exactly superimposed.

There was a comparatively sharp threshold strength of stimulation at which following impulses would suddenly appear; in preparations with several such impulses, each one had its own threshold. Often as the strength was slowly increased, one would appear intermittently, and on further increase would

become regular, finally becoming intermittent again as the delay became longer. In such cases it is possible that the discharge would only occur when two or more fibres of rather differing thresholds were excited.

Fig. 8. Double nerve preparation. Following impulse showing increasing delay. The shock artefact is a diphasic spike whose main deflexion is downwards. Note the relative positions of the impulse and artefact at the beginning and end of the series. The two records are continuous.

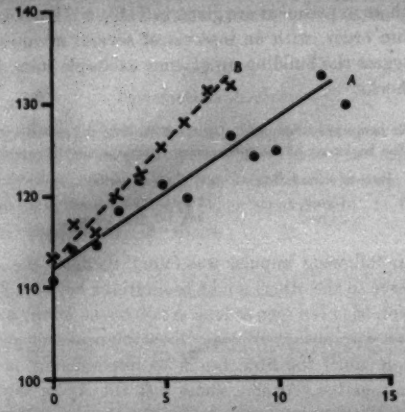

Fig. 9. Double nerve preparation. *A*, stimulation at 6/sec.; *B*, stimulation at 12/sec. Note that the two lines end at about the same height, and lie very much nearer one another than they would if the abscissa had been 'duration of stimulation', rather than 'number of shocks'. Ordinate: interval between shock artefact and following impulse in msec. (the 'delay'); abscissa: number of shocks after the beginning of the period of stimulation.

In seeking an explanation for the following impulses, four possibilities have suggested themselves, and it will be convenient to discuss their further properties under these headings:

(1) *Direct conduction from one nerve trunk to the other.* This would seem to be the most obvious explanation, were it not for the following difficulties:

(*a*) The long delay. The conduction velocity of A_2 fibres is, according to Fessard & Segers (1943), about 10 m./sec., and of the slow about 1 m./sec.; the

total length of nerve involved was not more than 2 cm. Therefore, assuming that the conduction velocity remains constant over the whole length of nerve, the delay would be of the order of 2–20 msec., i.e. far too short to account for the majority of following impulses. Also, while direct conduction might account for the long delay of the slow impulses, it can hardly account for it in the fast ones, unless we are to assume that a fast fibre in one trunk can connect directly with a slow fibre in another. This remains a possibility, since Weddell (1941) has shown that in human skin a single end-organ receives branches from at least two fibres, as well as a fine non-medullated 'accessory' fibre; there is, however, no mention of anything comparable in the frog. Alternatively, as Tower (1940) has suggested, the branches of a single fibre might become progressively finer and finer, and its conduction velocity slower, towards the peripheral part of its distribution, so that those impulses which were conducted through the outer branches would have a relatively long delay. However, it would be difficult to account for a delay of 100 msec. as has frequently been observed, on either of these suggestions.

(*b*) The fact that the delay usually increased as stimulation proceeded.

(*c*) In those preparations where a whole series of following impulses was found, there was no apparent relation between their conduction velocities, delays and thresholds. Often, however, impulses with a very short delay required a considerably stronger shock than the others.

(*d*) Reversal of the electrodes, i.e. recording from the nerve that had previously been stimulated and vice versa, caused the appearance of a completely different set of following impulses. Sometimes they differed in number, conduction velocity, delay, and rate of increase of delay.

Despite these objections, it is possible that a few following impulses can be explained by direct conduction, but this only applies to a small group found in some preparations, which did not disappear on reversal of the electrodes, and which had a constant delay, usually below 10 msec., and a low threshold.

(2) *Electrotonic spread of current.* The threshold for the response was usually high, and it is possible that sense-organs in the skin were being stimulated directly by electrotonic spread of the shock down the nerve. The delay would then correspond to the utilization time. If this were so, it would be expected that the delay would decrease as the strength of shock increased, though this was not found to be the case. A further difficulty in this explanation is that when a group of following impulses was found, there were often slow impulses preceding some of the fast ones. This is illustrated in Fig. 10; immediately following the shock artefact, which is a diphasic spike, there are two fast impulses with a delay of only 3 or 4 msec.; then comes another fast spike at 15 msec. delay, followed by three or four slow, and a large fast one at 60 msec.; finally there are about two more slow impulses. Now if all these were caused by electrotonic spread, the stimulation of all would be simultaneous, and the

slow impulses would hardly be expected to appear before the fast. Both this and the preceding view finally become untenable, however, when account is taken of the random discharges, which sometimes accompanied the more regular ones, and were their counterpart at faster rates of stimulation.

(3) *Contraction of smooth muscle in the skin.* This might be responsible for the random discharges, but when these became regular, it is scarcely likely that such muscle could have been contracting in time with the stimulus at rates up to 20/sec. According to Ecker (1889), the dorsal skin contains a large amount of smooth muscle, whereas the abdominal skin contains very little. The experiments were accordingly repeated on abdominal skin, and the same results obtained. In addition, stimulation of the dorsal roots, before and after cutting all the sympathetic rami, had the same effect as stimulation of a cutaneous nerve. Thus it is unlikely that any known motor nerves are responsible, and mechanical causes can be eliminated.

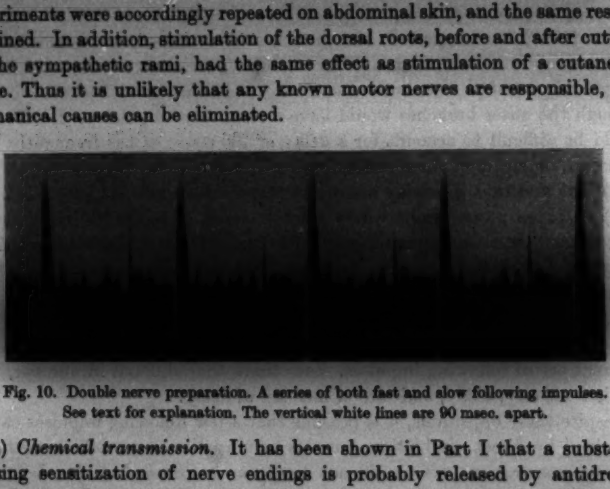

Fig. 10. Double nerve preparation. A series of both fast and slow following impulses. See text for explanation. The vertical white lines are 90 msec. apart.

(4) *Chemical transmission.* It has been shown in Part I that a substance causing sensitization of nerve endings is probably released by antidromic stimulation. It is not inconceivable that this may be released in sufficiently high concentration to cause other endings to fire off spontaneously. The threshold for sensitization was not always the same as that for the spontaneous discharges, and this looks like a serious objection to the suggestion that the two are related. However, those endings whose sensitivity was tested by the weight were not necessarily the same as those which fired off spontaneously, and it is possible that the occasions on which the two phenomena were not found together, can be ascribed to incorrect placing of the weight. At low rates of stimulation, it is possible that the concentration of this released substance may rise and fall sufficiently rapidly to allow the ending to respond in time with the stimulus.

This conclusion may seem surprising, but it is put forward tentatively as the only alternative left. Suppose we consider a single ending surrounded by endings from another nerve trunk, which is being stimulated antidromically.

As the strength of the shock increases, more endings will begin to release the sensitizing agent, and its excitability will rise, until it begins to fire off spontaneously; the frequency of discharge will then depend on its degree of adaptation, and the concentration of sensitizing agent in that region. The latter will not be constant, but, if it is assumed that the substance is released in small quanta as the separate nerve impulses arrive, it will undergo small variations at the frequency of stimulation. If sufficient neighbouring fibres are stimulated, these variations may cause the ending to fire off at the same frequency, or some submultiple of it. All the time, the fibre will have been adapting, a process which normally shows itself as a decreasing frequency of discharge; however in this case, the ending is being 'driven' at a fixed frequency, and adaptation will appear as an increasing delay. That this is the correct interpretation of the increasing delay, is suggested by the fact that it seems

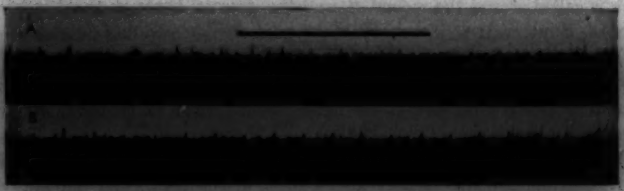

Fig. 11. Double nerve preparation. A slow following impulse with a delay of about 70 msec. The skin had previously been made to discharge spontaneously by histamine, and antidromic stimulation makes this become regular. The two records are continuous.

to depend more on the number of stimuli, than on the length of time for which stimulation has proceeded (cf. Fig. 9). As the delay increases, the system appears to become unstable, and only occasional random impulses are discharged from the ending, which is now nearly completely adapted.

It is suggested that this hypothesis accounts adequately for the sequence which has so often been observed, i.e. an intermittent following impulse, becoming regular as the stimulus strength is increased, and finally becoming intermittent again as the delay gets longer. Fig. 11 actually shows a random discharge becoming regular as an external frequency is imposed. A large slow spontaneous discharge had been produced by histamine; a nerve was then stimulated, and it can be seen that the impulses begin to follow the shock. In another preparation the excitability was so raised, that at each shock one fibre would respond with a burst of impulses at high frequency. Both of these are in agreement with the hypothesis, as is also the great variation in the types of response found in different preparations.

Stimulation with double shocks

There are obvious analogies between the behaviour of the preparation which has just been described, and that of a synapse. Experiments have therefore been performed to see whether it is possible to demonstrate in it anything corresponding to facilitation, using two shocks following one another at a short interval. Double shocks were provided by two induction coils, whose primary circuits were broken by a spring contact breaker. Preparations were found in which a following impulse appeared at the first shock when tested with the ordinary repetitive stimulator; each induction coil was then set so that a single shock would give rise to a following impulse, and several series of records were taken of the response to double shocks, with various intervals between the two. The observations were made in a random order with a pause of about a minute between each. The results are summarized below: for convenience the first shock is called *A* and the second *B*, and the interval between them is known as the *A–B* interval:

(1) Each shock had its appropriate following impulse at all *A–B* intervals above a certain minimum. This minimum varied between 7 and 3 msec. in different preparations; the latter figure means that there were following impulses at a frequency of over 300/sec.

(2) The impulse excited by *B* always had a longer delay than that excited by *A*. The increase was usually about 10 % of the total, and did not vary significantly with *A–B* intervals below 40 msec. (the longest used).

(3) If *A* failed to excite a following impulse, and *B* was successful, then the delay to *B* was the same as that usually found for *A*. In other words, the increase in delay is a consequence, not of the stimulus, but of the actual discharge of an impulse.

(4) The delay for the first following impulse remained approximately constant for different *A–B* intervals, though in some preparations there was a tendency for it to decrease when the interval was less than about 7 msec. The decrease was never more than 2 or 3 msec.

(5) Sometimes the response to *B* would consist of two following impulses, but it is not known whether they were in the same fibre. The occurrence of this second impulse bore no apparent relation to the *A–B* interval.

A protocol illustrates some of these points.

The delay to stimulation by single shocks was 34 msec. With double shocks and *A–B* intervals above 7 msec., the delay to *A* varied between 35 and 33·5 msec., and to *B* between 38 and 36·5 msec. With *A–B* intervals of less than 7 msec., the delay to *A* varied between 32 and 30 msec., and there was no response to *B*.

The results afford good confirmation of those obtained with repetitive stimulation, particularly in showing that the rate of increase of delay does not primarily depend on the frequency of stimulation (cf. Fig. 9). They also show

that if any facilitation takes place, as is suggested by the results under heading (5), it does not involve a change in delay that is significantly measurable, using the present technique.

The nerve fibres responsible

One curious fact is that fast fibres seem to be concerned in all these effects, just as much as the slow ones. The function of A_δ fibres is unknown, but they will respond to pressure, and Fessard & Segers (1943) describe them as the only ones responding to stretch. It is possible that they correspond to the fast group of pain fibres in man, but there is no evidence for it, and it does not seem very likely. Attempts have been made to find out which fibres were being stimulated, in three ways; first by threshold measurements. In most experiments the shocks used were large, indeed sometimes so large as to damage the fast fibres, and when applied to the cut peripheral end of another cutaneous nerve, were just about sufficient to cause a violent reaction indicative of pain. Secondly, it is possible to record the action potentials from an uncut cutaneous nerve during stimulation of a dorsal root. The pathway is sufficiently long for the different groups of impulses to have become separated, and it was found that the threshold at which following impulses appeared, was about that at which the slowest group of fibres was stimulated. The result was not invariable, however, and it was sometimes found that spontaneous discharges would start with very weak shocks. Finally some experiments were done with cocaine, which according to Gasser & Erlanger (1929), blocks conduction in the slow fibres before the fast; 0·3% cocaine regularly and rapidly abolished any spontaneous discharge, though the fast response to tactile stimulation remained unchanged. The effect was reversed after washing for about 20 min. It therefore seems probable that the slower group of fibres is the main one concerned.

The effect of drugs

Neoantergan. Since histamine was found to be such a powerful sensitizing agent in some preparations, the effect of this specific anti-histamine was investigated. When placed on the surface of the skin in a concentration of 1 in 1000, it caused disappearance of a following impulse in 3 min., though previously the response had been constant for 45 min. When placed subcutaneously, it had the same effect in 1 min., in a concentration of 1 in 10,000. If several following impulses were present, they would first become intermittent, and then drop out one by one. In high concentration neoantergan will act as a local anaesthetic, and this effect was sometimes observed, but in the majority of cases the spontaneous discharge disappeared long before the skin ceased to respond to tactile stimulation (but cf. cocaine, above). After soaking in Ringer for half an hour, the response would return, sometimes immediately, and sometimes gradually after prolonged stimulation. When first placed on the skin, it often caused a slight spontaneous discharge of slow impulses.

Acetyl choline usually had no effect at all, but once or twice there was a slight spontaneous discharge at concentrations of 1 in 100.

Atropine had no effect at all on following impulses in concentrations from 1 in 1000 to 1 in 1,000,000.

Prostigmin had no effect.

Sodium oxalate. It has been suggested that the increasing delay of following impulses might be explained as increasing adaptation. Talaat (1933) has shown that irrigation of the frog's skin with Ca^{++}-free Ringer, or oxalate, decreases the adaptation rate of the sensory receptors, and eventually causes them to

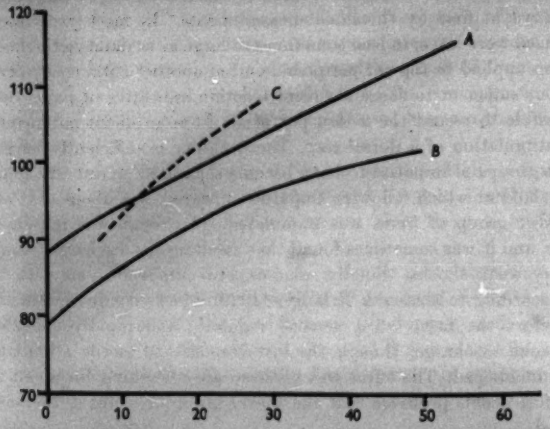

Fig. 12. Ordinate: delay of following impulse in msec.; abscissa: number of shocks after the beginning of the period of stimulation. *A*, normal response; *B*, Ca^{++}-rich after 15 min. in 0·6 % sodium oxalate solution; *C*, after 30 min. in Ca-rich Ringer.

fire off spontaneously. Some experiments have therefore been performed, in which a double nerve preparation was irrigated first with Ringer, then with 0·6 % sodium oxalate and 0·7 % sodium chloride solutions for about 15 min., and finally with Ringer containing ten times the normal concentration of calcium. At 5 min. intervals, the preparation was stimulated antidromically, and records taken of the following impulses. For each period of stimulation the delay was then plotted against the number of shocks.

In all the experiments there was a decrease in the average delay for the successive periods of stimulation, during irrigation with oxalate. After about half an hour in the Ca^{++}-rich Ringer, the delay began to increase again, but the experiments were not continued long enough for it to return completely to normal. These changes in delay varied between 10 and 20 % of the whole, but

do not necessarily represent the maximum that could be obtained. While the oxalate was on the skin, there was a gradually increasing spontaneous discharge in both fast and slow fibres, which tended to obscure the following impulses. This naturally limited the experiments. During any one period of stimulation, however, the delay increased as usual. Sometimes the rate of increase was slower than normal in oxalate, and faster in Ca^{++}-rich Ringer; in other experiments there were no significant changes. These points are illustrated in Fig. 12. In addition, the following impulses tended to become intermittent and disappear slightly sooner after oxalate than normally, but this may merely have been the result of a general deterioration of the preparation.

DISCUSSION

Perhaps the most striking conclusion to be drawn from these experiments is the close parallel between human and frog's skin. As Walshe (1942) has pointed out, however, the refinement of sensory function in the higher animals is not so much the result of better sensory apparatus, but because better use is made of the information received. Similar experiments have not yet been done on mammals, but Foerster (1925) used something analogous to the double nerve preparation, when he stimulated dorsal roots cut centrally in some of his patients; they complained of a burning pain in the skin, which was abolished by section of the nerve that, overlapping, supplied the same area. Lewis describes the same effect on stimulating cutaneous nerves blocked centrally by a local anaesthetic.

TABLE 5

Human	Frog
Antidromic stimulation causes hyperalgesia	Antidromic stimulation causes sensitization
Injury causes local redness and tenderness	Injury causes sensitization (Echlin & Propper, 1937)
'Antidromic vasodilation'	Vasodilation in tongue
Slow rise and long continuation of pain	Ditto for slow impulses (Hogg, 1935)
Foerster's experiments	Spontaneous discharges in double nerve preparations

The similarities between these results and those obtained on human skin are emphasized in Table 5, and while it is not at present safe to assume that the mechanisms are identical, they do demand a similar sort of explanation. To account for his observations, Lewis (1942) postulated the existence of a special nerve plexus, the nocifensor system. The question has recently been discussed by Walshe, who concludes that it is almost certainly the small fibre plexus serving pain sensation. This is in agreement with the results described in this paper, which suggest that the slow fibres are responsible. Lewis also showed, mainly by experiments involving occlusion of the circulation, that the effects which he observed were caused by the release of one or more chemical agents. The present experiments point to the same conclusion.

There is no indication as to the nature of the substance released, and no experiments have been done to investigate this. Lewis seems to postulate two distinct substances, responsible for the triple response and hyperalgesia respectively. He rejects H-substance, which causes the triple response, as causing the latter, since histamine pricked into the skin produces itching, and not pain. But as Sanders (1947) has pointed out, this objection is largely invalidated by the conception of itching as subthreshold pain. Further, to suggest, as Lewis did, that the itching associated with the triple response is caused by the release of H-substance, is useless without postulating also a special set of nerves, whose only functions are to react to H-substance, and to convey that sensation. In fact, it is possible to obtain pain by injection of histamine in sufficiently high concentration, and although this is higher than the natural content of skin, and so was not used by Lewis, there is no reason why the *local* concentration should not rise as high. Experiments by Feldberg & Emmelin (1947) on the mechanism of the nettle sting suggest that acetylcholine must also be present to cause pain, but the absence of any action by this drug, either with or without histamine, does not support this view in the frog.

All the phenomena described by Lewis were very similar, and seem to differ only in degree, and it is suggested that they were all caused by release of the same substance. In view of the similarities between them and observations in the frog, it is probable that the same substance is responsible there too, especially since it is possible to extract an active H-substance from the skin. The pharmacological experiments, particularly those with neoantergan, must be treated with caution, however, since they are notoriously difficult to interpret; they should not be regarded as positive evidence for such a chemical agent, but merely as confirming what would be expected, if it did exist.

In some preparations sensitization could not be produced by any method. Similarly Echlin & Propper (1937) found that scraping the skin was only successful in producing it in about 50% of their frogs, and Lewis mentions the non-occurrence of hyperalgesia in quite a large proportion of human subjects. There are two possible explanations of this: either the skin does not contain the active principle, or the sensory nerve endings are less accessible or reactive to it. In view of the large differences in the response to histamine or skin extract, the latter explanation seems the most likely, but even preparations which were quite inactive to any drugs, would often give spontaneous discharges after antidromic stimulation. This is difficult to reconcile with either suggestion.

It should here be emphasized that the hypothesis put forward to account for the spontaneous discharges is by no means completely satisfactory, and it is possible that they cannot be assigned to any one cause alone. Some form of 'ephaptic' transmission, such as has been found in a cut or crushed mammalian nerve, still remains a possibility, though such phenomena are rare in frogs. Broadly speaking, however, this explanation raises the same objections as were

found against direct conduction. Some difficulties face all explanations. For example, in the experiments with oxalate, according to the present hypothesis, a change in the rate of increase of the delay of the following impulse would have been expected, rather than a change in its average delay, as was observed. Or again, sometimes the delay would show completely unpredictable variations, and was often shorter after a single shock than after the first part of a double one. In fact, this paper must be regarded only as a preliminary classification of the phenomena, which will probably appear more explicable as investigation proceeds.

SUMMARY

1. Investigation has been made of the sensitization described by Echlin & Propper after scraping the frog's skin.

2. The same effect has been seen after antidromic stimulation of cutaneous nerves. By the use of different kinds of preparation this has been shown to be caused by the release of some substance at the nerve endings.

3. Antidromic stimulation of the glossopharyngeal nerve has been shown to cause reddening of the tongue.

4. Spontaneous discharges have been seen during and after stimulation. It is suggested that they are caused by release of the same substance.

5. During antidromic stimulation of one cutaneous nerve, impulses have been found coming down a neighbouring nerve in time with the stimulus. It is suggested that the impulse is transmitted from one nerve to the other chemically.

6. The action of various drugs has been tried on the skin, and it is suggested that a histamine-like substance is responsible for all these effects.

7. The findings have been compared with those of Lewis working on human skin.

The author wishes to thank Dr B. H. C. Matthews for constant help and advice; also the Medical Research Council, for a training grant which enabled the work to be undertaken.

REFERENCES

Echlin, F. & Propper, N. (1937). *J. Physiol.* **88**, 388.
Ecker, A. (1889). *The Anatomy of the Frog* (trans. by G. Haslam), p. 369. Oxford: Clarendon Press.
Feldberg, W. & Emmelin, N. (1947). *J. Physiol.* **106**, 440.
Feng, T. P. (1933). *J. Physiol.* **79**, 103.
Fessard, A. & Segers, M. (1943). *C.R. Soc. Biol., Paris*, **137**, 212.
Foerster, O. (1925). *Festschrift. f. Rossolimo.* Cited by Lewis in *Pain*, p. 80.
Gasser, H. S. & Erlanger, J. (1929). *Amer. J. Physiol.* **88**, 581.
Grant, R. T. & Jones, T. D. (1929). *Heart*, **14**, 339.
Hogg, B. M. (1935). *J. Physiol.* **84**, 250.
Lewis, T. (1942). *Pain.* New York: Macmillan.
Sanders, F. K. (1947). *Ann. Rev. Physiol.* **9**, 553.
Talaat, M. (1933). *J. Physiol.* **79**, 500.
Tower, S. S. (1940). *J. Neurophysiol.* **3**, 486.
Walshe, F. M. R. (1942). *Brain*, **65**, 48.
Weddell, G. (1941). *J. Anat., Lond.*, **75**, 441.
Woollard, H. H., Weddell, G. & Harpman, J. A. (1940). *J. Anat., Lond.*, **74**, 413.

J. Physiol. (1950) 111, 215–230 612.819.911

THE VASOMOTOR RESPONSES DUE TO ELECTRICAL STIMULATION OF THE SINUS AND VAGUS NERVES OF THE CAT AND THEIR MODIFICATION BY LARGE DOSES OF SODIUM PENTOBARBITAL (NEMBUTAL)

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It is generally accepted that electrical stimulation of the central ends of the buffer nerves, that is the carotid sinus and aortic nerves, causes a fall in arterial blood pressure. Since chemosensory pressor fibres are present in both nerves, it should be possible to obtain pressor responses by electrical stimulation if conditions are made favourable for a preponderance of the pressor element. This is readily done in both nerves by abolishing the barosensory depressor response by means of large doses of sodium pentobarbital (nembutal), and in the carotid sinus nerve by so varying the strength of the stimulus as to activate more effectively the chemosensory component than the barosensory. Similarly, the depressor response obtained by administering strong electrical stimuli to the central ends of the vagi can be converted to a pressor one by overdosage with nembutal.

The more important of these findings have already been presented at meetings of the Physiological Society (Douglas, Innes & Kosterlitz, 1948, 1949).

METHODS

Cats were anaesthetized with nembutal (40–50 mg./kg., intraperitoneally) or urethane (25% (w/v) solution, 1–1·5 g./kg., intraperitoneally).

In order to minimize the effects on the arterial blood pressure of changes in blood gas tensions and intrathoracic pressure following stimulation or inhibition of the respiratory centre, an open pneumothorax was applied in most experiments. In a few early experiments the pneumothorax was effected by intubation by means of a rubber tube through the diaphragm. In most experiments, however, a bilateral open pneumothorax was applied by resection of a rib to produce an opening in each side of the chest, measuring about 1·5 cm. in diameter. To ensure a pulmonary ventilation similar to that prior to the pneumothorax, the lungs were inflated through a tracheal cannula by means of an 'Ideal' pump so that in normally anaesthetized animals respiratory movements of the chest were not suppressed but were just present. These respiratory movements were, of course, quite independent of the rhythmic inflations of the lung produced by the pump and could in no way interfere with pulmonary ventilation which was maintained at a constant rate throughout the experiment. Tracings of the spontaneous respiratory movements were obtained by means of a

stethograph and tambour, and gave a good record of excitation or inhibition of the respiratory centre after stimulation of the buffer nerves and of depression of the centre after large doses of nembutal. This procedure was not possible in some animals, in which, obviously due to the anaesthetic, the respiratory centre was found to be too depressed before the application of the pneumothorax. In these animals spontaneous respiratory movements were absent when the artificial ventilation of the lungs was kept at a level adequate to prevent cyanosis.

The arterial blood pressure was recorded, with heparin as anticoagulant, in a femoral artery or in the left carotid artery, when the left sinus nerve was divided.

The aortic nerve was identified as a small nerve leaving the vagus and joining the superior laryngeal nerve, and the sinus nerve was readily found after ligature and section of the external carotid artery and dissection of the glossopharyngeal nerve which was cut about 1 cm. distally to the entry of the sinus nerve. Great care was taken to free the sinus nerve from surrounding tissue in order to prevent spreading of the stimulating current. For stimulation, the sinus nerve was placed on shielded silver—silver chloride electrodes embedded in Perspex. No signs of spreading of current were ever observed. Stimuli 8–10 times greater than the strongest used in the experiments described in this paper were required to produce such spreading. With such, and even stronger stimuli, ligation of the sinus nerve centrally to the stimulating electrodes abolished all effects on the vasomotor and respiratory centres, showing that current spread, even if it occurred, was not responsible for the observed phenomena. In almost all experiments both vagi and the left sinus nerve were cut while the right sinus nerve was left intact. Thus, the central ends of the vagus and aortic nerves were stimulated, while the right sinus nerve was usually stimulated intact. This was done to preserve the delicate nerve for the whole duration of the experiment which sometimes lasted several hours. In a small number of experiments the right sinus nerve was stimulated before and after section of the nerve close to the carotid sinus. No significant difference was observed.

Cold block was applied to the sinus nerve by placing the nerve on a U-shaped fine copper tube through which brine at about -8 to $-10°$ C. was allowed to flow. The nerve in contact with the U-tube was frozen in a very short time. It was thawed by stopping the flow of brine and irrigating the nerve with warm Ringer-Locke solution.

Decerebration was carried out in the usual way. In experiments where the medulla was sectioned, the cerebellum was first removed. Sections of the medulla were made with a single sweep of a decerebrator. As each slice of the medulla was removed, it was transfixed with a needle and the individual slices were built up on the needle to reproduce the whole. The responses to electrical stimulation of the buffer nerves were tested after each section.

Three stimulators were used for electrical stimulation: (1) The stimulator designed by Whitfield (1946), consisting essentially of condenser discharges through a neon tube. The duration of the exponentially declining pulse is approximately 0·3 msec. and its physiological efficacy corresponds to a rectangular pulse of approximately 0·03 msec. duration. (2) A thyratron stimulator of conventional design, the exponentially declining pulse lasting between 0·4 and 3·5 msec. corresponding to rectangular pulses lasting between approximately 0·04 and 0·35 msec. (3) The Ritchie-B.N.I. (Walter & Ritchie, 1945) 'square wave' stimulator, with rectangular pulses lasting between 0·02 and 10 msec. By a minor modification it was possible to obtain frequencies additional to those provided in the instrument. The frequency, pulse duration and voltage (base-peak) of the stimuli were confirmed by continuous monitoring by means of a cathode-ray oscilloscope. Bipolar platinum electrodes were used for stimulation of the vagi and shielded bipolar silver-silver chloride electrodes for the sinus and aortic nerves.

Since in the experiments to be described, the voltage and the duration of the stimulating pulses vary, it will be impossible to define the stimuli in terms of strength (voltage) alone. For this reason, the stimuli will be characterized by the product voltage × duration (Vt).

The results presented in this paper are based on experiments on over eighty cats.

RESULTS

Changes in arterial blood pressure and respiration on electrical stimulation of the sinus nerves in nembutalized and urethanized cats

On stimulation of the right sinus nerve, with both vagi and the left sinus nerve cut, pressor or depressor responses were obtained in more than forty cats, depending on the Vt (voltage × pulse duration) of the stimuli. In many animals anaesthetized with nembutal or urethane, three different responses were obtained, viz. a depressor response with stimuli of low Vt, a pressor response with stimuli of slightly higher Vt, and again a depressor response with stimuli of considerably higher Vt. The first response was present in about half the cats, the second in all animals and the third in most animals. As was to be expected, relatively high voltages were required when very short pulses were used, while with longer pulses the voltages were considerably lower. For example, with a rectangular pulse lasting 0·02 msec., 3 V. caused a depressor response, 8 and 10 V. pressor responses, and 30 V. again a depressor response (Fig. 1). In another cat, with rectangular pulses lasting 0·1 msec., 0·5 and 1 V. gave pressor responses, 2 V. a poorly maintained rise of blood pressure followed by an after-fall, 3 V. a biphasic response consisting of a rise followed by a transient fall, 4 V. a fall of blood pressure after a considerable lag and 5 V. a marked depressor response (Fig. 2). If, for a given voltage and pulse duration, the response was pressor, increasing the Vt by prolonging the pulse duration also led to a depressor response. These results were in no way influenced by the type of stimulator used.

No quantitative comparisons could be made between the products of voltage and pulse duration required to obtain similar responses in different animals, and, even in the same animal, this was rarely possible if some time had elapsed between the tests. The experimental conditions, particularly the depth of anaesthesia and the resistance of the nerve, could not be kept sufficiently constant to permit a quantitative evaluation of simultaneous variations in voltage and pulse duration. There is, however, no doubt about the validity of the qualitative findings: increasing the Vt of the stimulus, either by increasing the voltage or the pulse duration, will almost always convert a pressor response to a depressor unless, as will be shown later, too much nembutal has been given.

The depressor response elicited by stimuli of low Vt was readily obtained only with weak pulses of very short duration. The range over which it was present was remarkably narrow, any slight increase in the voltage evoking a pressor effect. The fall in blood pressure was always slight, much smaller than that observed in the depressor responses with stimuli of high Vt. It is also to be noted that the pressor responses were obtained over a wide range and that, with stimuli of increasing Vt, there were responses intermediate between pressor and depressor (Fig. 2).

When spontaneous respiratory movements were present, stimulation of the sinus nerve with stimuli of medium and high *Vt* always caused an increase of these movements, whether the vasomotor response was pressor or depressor (Fig. 2). The maximum respiratory activity was achieved before the pressor

Fig. 1. Cat. Urethane, 1·5 g./kg. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left carotid artery in mm. Hg. Time 5 sec. Stimulation of right sinus nerve, 0·02 msec., square wave, 70 cyc./sec. *A*, 3 V.; *B*, 8 V.; *C*, 10 V.; *D*, 30 V.

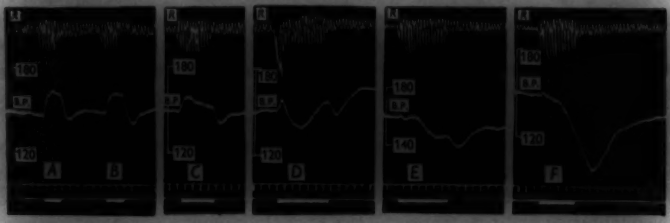

Fig. 2. Cat. Nembutal, 40 mg./kg. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. *R*, respiratory movements, inspiration downwards. B.P. in left carotid artery in mm. Hg. Time 5 sec. Stimulation of right sinus nerve, 0·1 msec., square wave, 70 cyc./sec. *A–F*: consecutive stimuli over a period of 8 min. *A*, 0·5 V.; *B*, 1 V.; *C*, 2 V.; *D*, 3 V.; *E*, 4 V.; *F*, 5 V.

response became purely depressor. On the other hand, stimuli of low *Vt* giving a depressor response did not influence the respiratory centre. When spontaneous respiratory movements were absent, respiratory movements often reappeared on stimulation of the sinus nerve with stimuli of medium or high *Vt*.

The effects of changes in frequency of the stimulus have not been examined systematically; it has been found, however, that changes in frequency caused a 'reversal' of the response only when the stimulus was of such a *Vt* that the

response was on the borderline between pressor and depressor. For instance, square wave stimuli of 0·02 msec. duration and 7 V. gave pressor responses at frequencies between 1·5 and 300 cyc./sec. When the voltage was raised to 30 V., frequencies below 20 cyc./sec. resulted in pressor, and frequencies over 70 cyc./sec. in depressor responses.

The fact that the choice of the anaesthetic, nembutal or urethane, had no influence on the results obtained in cats given the normal dose of anaesthetic is of some importance, since, as will be shown later, overdosage with urethane, unlike nembutal, does not convert depressor into pressor responses.

The effect of overdosage of nembutal on the response to electrical stimulation of the sinus, vagus and aortic nerves, and the action of picrotoxin

The sinus nerve. It was shown that electrical stimulation of the sinus nerve with a strong stimulus of short duration led to a marked depressor response in the cat given the normal anaesthetic dose of about 40–50 mg./kg. nembutal. It will be suggested in the discussion that such a stimulus activates three types of fibres, viz. large barosensory, small chemosensory and small barosensory fibres, the net result being a fall in blood pressure. If, however, the animals had received a second dose of nembutal of half the amount required for the initial anaesthesia, then strong stimuli of the type just described did not result in a fall of blood pressure, but led to pressor responses (Fig. 3). These reached a maximum in about 45 min. after the additional dose of anaesthetic and lasted for an hour or more without decrement. During this period the blood-pressure level was lower than before the administration of the second dose of nembutal. Sometimes, before the pressor response was fully developed, pressor responses with an after-fall (Fig. 4*B*) or biphasic responses (Fig. 4*C*), were observed. Once the pressor responses were fully developed, no variation in voltage or pulse duration was able to elicit depressor responses. The pressor responses were remarkably uniform with no variations in the heart rate. A further administration of nembutal eventually led to a disappearance of all vasomotor responses to sinus nerve stimulation.

The response of the respiratory centre to sinus nerve stimulation in the over-nembutalized animal persisted in twenty-one out of thirty-four cats. The response, however, was smaller than that found before the second dose of nembutal (Fig. 5).

When the animals were allowed to recover in the course of a few hours from the effects of the overdosage, still remaining fully anaesthetized, the pressor response slowly diminished and, eventually, the response to sinus nerve stimulation was again depressor as in the normally nembutalized cat (Fig. 3). Biphasic responses similar to those described after a second dose of nembutal were often observed. Injection of more nembutal at this stage again led to a pressor response.

The return of the depressor response could be very markedly accelerated by administration of picrotoxin, which is extensively used in the clinical treatment of barbiturate poisoning. Intravenous injection of approximately 1–1·5 mg./kg.

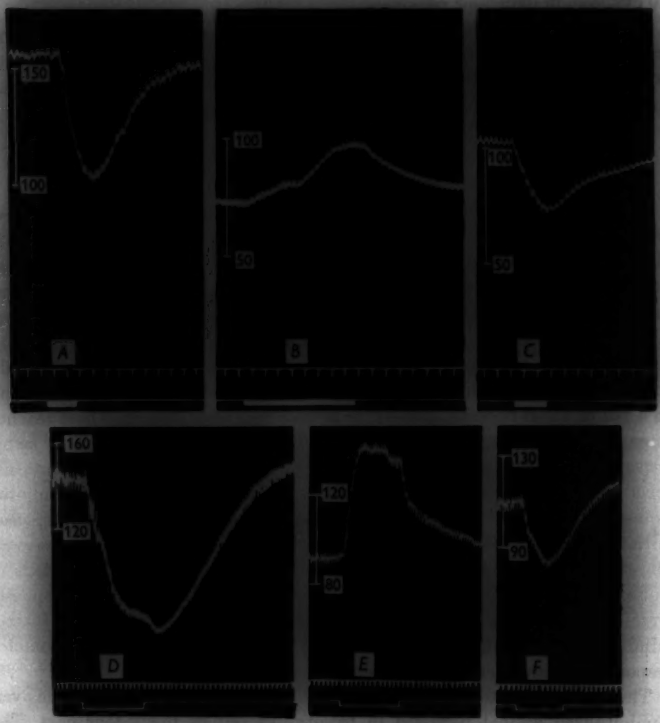

Fig. 3. Cat. Nembutal. Both vagi and left sinus nerve cut. Open pneumothorax by intubation through diaphragm. Artificial respiration. B.P. in left carotid artery in mm. Hg. Time 5 sec. Stimulation of right sinus nerve with neon tube stimulator, 40 V., 30 cyc./sec. *A*, after 50 mg./kg. nembutal; *B*, after 80 mg./kg.; *C*, 3 hr. later; *D* another cat, after 43 mg./kg. nembutal; *E*, after 83 mg./kg.; *F*, after 1·1 mg./kg. picrotoxin.

picrotoxin led within 5–10 min. to the reappearance of the depressor response, and usually to a rise of blood pressure (Fig. 3). Biphasic responses were sometimes observed in the transition period. Injection of nembutal at this stage re-established the pressor response.

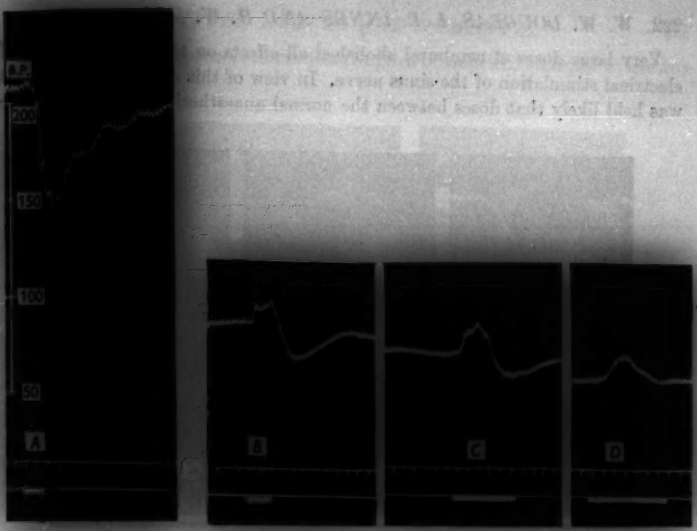

Fig. 4. Cat. Nembutal. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left carotid artery in mm. Hg. Time 5 sec. Stimulation of right sinus nerve with neon tube stimulator, 20 V., 30 cyc./sec. *A*, after 40 mg./kg. nembutal; *B*, 12 min. after additional 20 mg./kg.; *C*, 4 min. after *B*; *D*, 9 min. after *C*.

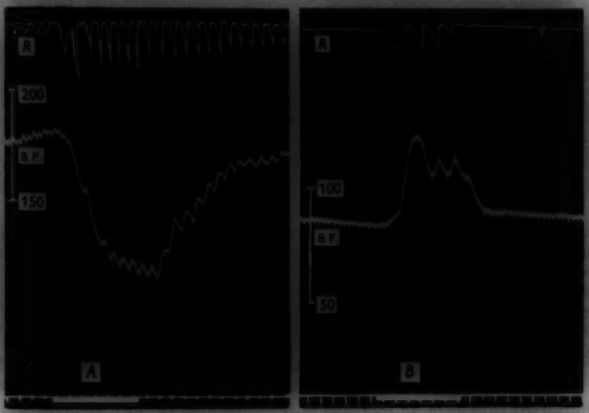

Fig. 5. Cat. Nembutal. Both vagi cut, both sinus nerves intact. Bilateral open pneumothorax. Artificial respiration. *R*, respiratory movements, inspiration downward. B.P. in femoral artery in mm. Hg. Time 5 sec. Stimulation of left sinus nerve with neon tube stimulator, 20 V., 30 cyc./sec. *A*, after 50 mg./kg. nembutal. *B*, 25 min. after additional 20 mg./kg. nembutal.

Very large doses of nembutal abolished all effects on the blood pressure of electrical stimulation of the sinus nerve. In view of this depressant action, it was held likely that doses between the normal anaesthetic dose and the dose

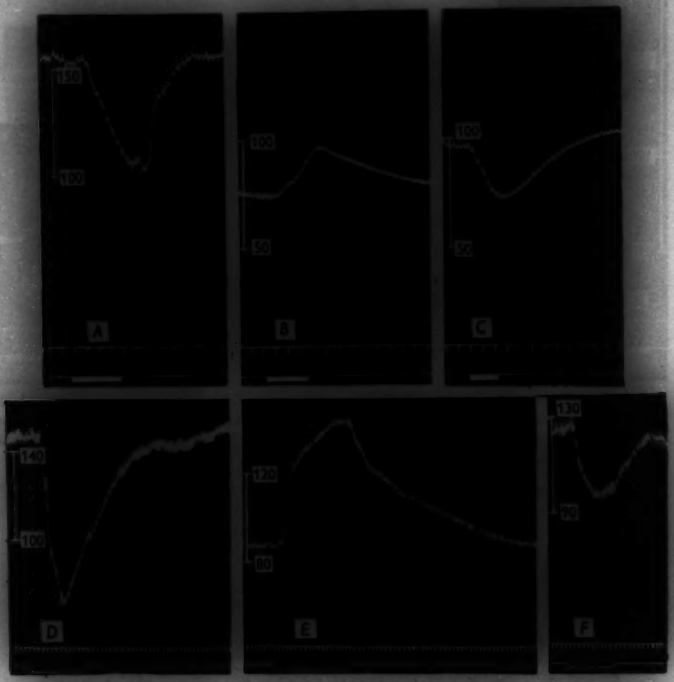

Fig. 6. Cat. Nembutal. Both vagi and left sinus nerve cut. Open pneumothorax by intubation through diaphragm. Artificial respiration. B.P. in left carotid artery in mm. Hg. Time 5 sec. Stimulation of right vagus nerve with neon tube stimulator, 40 V., 30 cyc./sec. *A*, after 50 mg./kg. nembutal. *B*, after 80 mg./kg. *C*, 3 hr. later. *D*, another cat, after 43 mg./kg. nembutal. *E*, after 83 mg./kg. *F*, after 1·1 mg./kg. picrotoxin.

causing complete absence of response would cause a rise in the threshold of both pressor and depressor reflexes. It was possible to observe in some over-nembutalized cats such an increase in the threshold of both reflexes so that in order to obtain depressor responses the strength of stimulus had to be increased. If, however, still more nembutal were given only pressor responses were obtained, no matter how strong the stimulus. In negative attempts still to

obtain depressor response, stimuli were applied of sufficient strength to destroy the nerve.

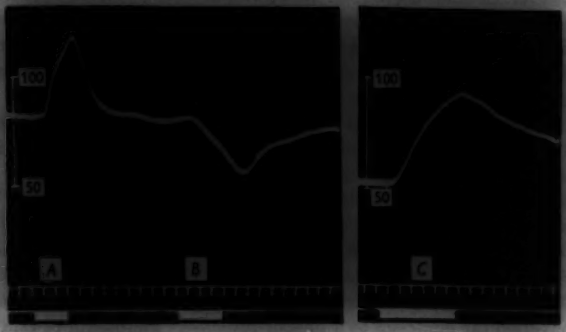

Fig. 7. Cat. Nembutal. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left carotid artery in mm. Hg. Neon tube stimulator, 40 V., 30 cyc./sec. *A*, right sinus nerve after 65 mg./kg. nembutal. *B*, right vagus nerve after 65 mg./kg. *C*, right vagus nerve after 80 mg./kg.

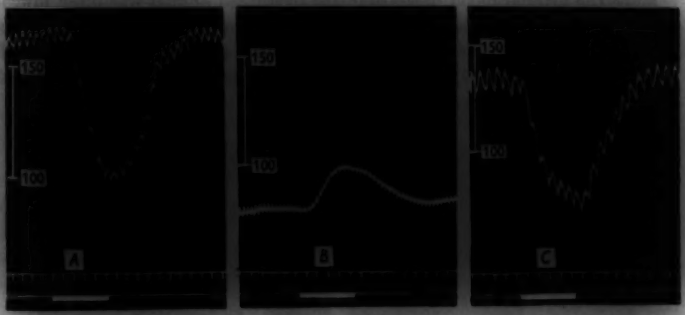

Fig. 8. Cat. Nembutal. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left femoral artery in mm. Hg. Time 5 sec. Stimulation of left aortic nerve, 1 msec., square wave, 10 V., 70 cyc./sec. *A*, after 45 mg./kg. nembutal. *B*, after 70 mg./kg. *C*, after 2·4 mg./kg. picrotoxin. (Respiratory waves are absent from *B* due to cessation of spontaneous respiratory movements during nembutal overdosage.)

The vagus nerve. In the cat, given the normal anaesthetic dose of nembutal, electrical stimulation of the central ends of the vagus nerves with a strong stimulus usually led to a marked depressor response. After a second dose of nembutal of about half the amount required for the initial anaesthesia,

this response became pressor. Spontaneous recovery, and recovery due to picrotoxin, did not show any difference from similar phenomena already described for the sinus nerve (Fig. 6). It was, however, obvious that the vagal reflexes were less sensitive to overdosage with nembutal than the sinus nerve reflexes, that is to say, with a suitable dose of nembutal normal depressor responses were still elicited by stimulation of the vagus while the sinus nerve response was pressor (Fig. 7).

With the strength of stimulus required to obtain a depressor response in the normally nembutalized cat an inhibition of respiration was observed. After the reversing dose, spontaneous respiration ceased and in thirteen out of thirty-four cats the stimulus previously causing inhibition of respiration now caused stimulation.

The aortic nerve. Stimulation of the central end of the left aortic nerve in three cats led to a fall in arterial blood pressure in the cat anaesthetized with nembutal (40–50 mg./kg.). Further injection of half the original anaesthetic dose converted the depressor to a pressor response. Picrotoxin re-established the depressor response (Fig. 8).

The effect of low blood pressure on the response to electrical stimulation of the sinus and vagus nerves

Since the arterial blood pressure in the overdosed animal was often low, it was necessary to test whether low arterial blood pressure could by itself 'reverse' the response. This is of importance in view of the results of McDowall (1925) and Wright (1932), who found that in animals with low arterial blood pressure pressor fibres may be active in the vagus nerve.

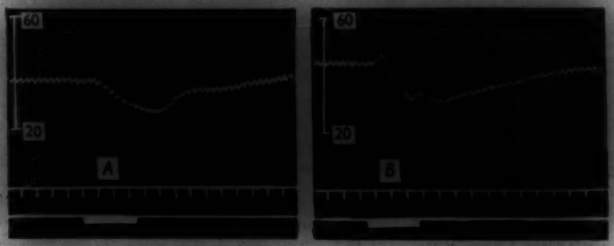

Fig. 9. Cat. Nembutal, 40 mg./kg. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left carotid artery in mm. Hg. Time 5 sec. Neon tube stimulator, 40 V., 30 cyc./sec. *A*, right vagus nerve; *B*, right sinus nerve.

Low pressures in cats given the normal anaesthetic dose of nembutal were obtained either at the end of lengthy experimental procedures (two cats), or after repeated bleedings (two cats). Two more cats were under urethane

anaesthesia, the low pressure being consequent upon the combined effects of operative interference and bleeding. In all these animals, stimulation of the vagus and sinus nerves resulted in depressor responses, even when the pressure was initially as low as 40 mm. Hg (Fig. 9). It is also of interest to note that administration of picrotoxin to cats overdosed with nembutal may raise the blood pressure without converting the pressor response to a depressor while, on the other hand, it may cause depressor responses to reappear without raising the blood pressure.

Effect of decerebration at various levels

In these experiments, cats were first given the normal anaesthetic dose of nembutal and the responses to stimulation of the vagus and sinus nerves tested. This was repeated after administration of the 'reversing' dose. Then decerebration was performed. In four out of five cats, stimulation of the buffer nerves after decerebration at the level of the superior colliculi gave pressor responses similar to those obtained before; picrotoxin still converted the pressor to depressor as in the animals with the brain intact (Fig. 10). In the fifth animal, the responses, pressor before decerebration, were depressor after. On administration of more nembutal, however, they again became pressor. Sectioning below the superior colliculi down to the entry of the ninth and tenth nerves did not affect the pressor responses nor the efficacy of picrotoxin in abolishing the nembutal reversal. This fact indicates that no centres higher than the medullary vasomotor centres are involved in the action of nembutal or picrotoxin on these vasomotor reflexes.

The traction test in nembutal overdosage

In cats with both vagi and the left sinus nerve cut, traction on the occluded right common carotid artery caused a fall in arterial blood pressure if the normal anaesthetic dose had been given, and a rise in blood pressure if the animal had been overdosed with nembutal. These effects were still present, although diminished, when the sinus nerve was frozen but were absent after severing the nerve. Traction of the cut sinus nerve in the overnembutalized cat gave a pressor effect similar to that observed after traction of the carotid artery with the nerve frozen (Fig. 11). At least part of the effect of the traction test is therefore due to mechanical stimulation of the axons in the nerve itself central to the frozen area. In nembutal overdosage such traction results in a rise of blood pressure, traction on the depressor fibres being ineffective.

The effect of overdosage of urethane

For the analysis of the effects of electrical stimulation of the sinus nerve with stimuli of varying *Vt* it was of importance to compare the results obtained on nembutalized cats with those obtained on cats given an anaesthetic which, even

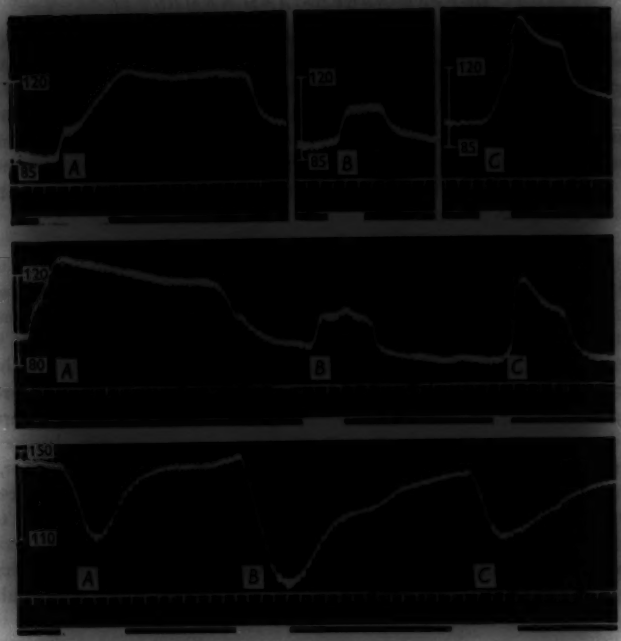

Fig. 10. Cat. Nembutal. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. B.P. in left carotid artery in mm. Hg. Neon tube stimulator, 40 V., 30 cyc./sec. *A*, left vagus nerve; *B*, right vagus nerve; *C*, right sinus nerve. I: after 78 mg./kg. nembutal. II: 16 min. later, after decerebration at level of superior colliculi. III: after 1·8 mg./kg. picrotoxin. (Signal for II *A* is missing.)

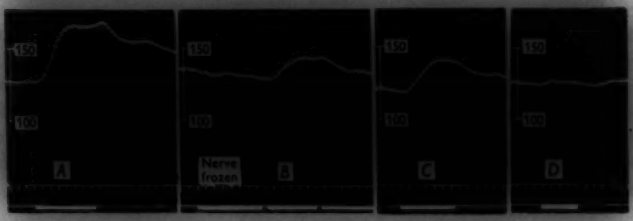

Fig. 11. Cat. Nembutal, 65 mg./kg. Both vagi and left sinus nerve cut. Bilateral open pneumothorax. Artificial respiration. Right common carotid artery occluded throughout. B.P. in left carotid artery in mm. Hg. Time 5 sec. *A*, traction applied to the right common carotid artery. *B*, repeated with the right sinus nerve frozen. Right sinus nerve cut between *B* and *C*. *C*, traction applied to the cut right sinus nerve. *D*, traction on the carotid artery repeated after sinus nerve section.

in large doses, did not lead to a 'reversal'. It was found that urethane fulfilled these conditions.

Cats were anaesthetized with urethane (1–1·5 g./kg. in 25% solution, intraperitoneally). When suitable stimuli had been selected which gave depressor responses on stimulation of the vagus and sinus nerves, the animals were given intravenously, at intervals, additional doses of one-quarter of the original anaesthetic dose. The magnitude of the responses tended to diminish as the anaesthesia became deeper, but there was no 'reversal' as in nembutal overdosage, even in cats which had received a total of three times the anaesthetic dose.

DISCUSSION

From studies of the action potentials of the sinus nerve it has been known for some time that it contains large barosensory and smaller chemosensory fibres (Bronk, 1931; Bronk & Stella, 1932; Heymans & Rijlant, 1933; Zotterman, 1935). In addition, Euler, Liljestrand & Zotterman (1939, 1941) adduced evidence that apart from the large barosensory fibres, the sinus nerve also contains small fibres which respond to variations in endosinusal pressure and are insensitive to chemical stimuli. They found that sometimes the large spikes due to activity of the large barosensory fibres were absent. If, in such preparations, they silenced the chemosensory fibres by hyperventilation with O_2, small spikes were observed when the arterial blood pressure was raised. Since these small spikes were very numerous, it was concluded that the small barosensory fibres might be of greater importance in the sinus pressure reflexes than the large ones. The amplitude of the action potentials was similar to that of the chemosensory fibres.

In the light of these findings, the following interpretation of the response of the sinus nerve to electrical stimulation suggests itself: weak stimuli of low *Vt* activate the large barosensory fibres with a low threshold, causing a fall in blood pressure. A small increase in the *Vt* of the stimulus also activates the chemosensory fibres with a slightly higher threshold, leading to a rise in arterial blood pressure as soon as the effect of the stimulation of the numerous chemosensory fibres overcomes that of the large barosensory fibres. A further rather considerable increase of the *Vt* of the stimulus at first intensifies the pressor and then successively diminishes it and converts it into a depressor response as more and more of the small barosensory fibres with a high threshold are brought into play. The magnitude of the stimulus required to activate the small barosensory fibres indicates that they are smaller than the chemosensory fibres. It also suggests that their threshold is of the order usually found in C fibres (Blair & Erlanger, 1933). If this is the case, the powerful stimulus required to activate them is in agreement with the findings of Gordon (1943), who stressed the difficulty of exciting vasomotor C fibres in mixed sensory nerves with brief condenser shocks.

The view that the pressor responses are due to stimulation of the chemosensory fibres is supported by the following facts:

(1) Stimuli of low *Vt*, which activate the large depressor fibres only, have no effect on the respiratory centre. Stimuli of medium *Vt*, which activate the large depressor and the small pressor fibres, yielding a pressor response, stimulate the respiratory centre as shown by the increase in respiratory movements. Stimuli of high *Vt* which stimulate also the small depressor fibres, yielding a depressor response, have little or no additional effect on the respiratory centre.

(2) After the 'reversing' dose of nembutal, the pressor responses due to stimuli of high *Vt* are in the majority of animals still accompanied by a stimulation of the respiratory centre.

The physiological significance of the two types of barosensory fibres is not clear at present. There will, however, be a considerable difference between the times taken for the conduction by the large and small fibres of barosensory impulses from the carotid sinus to the medulla. It is at least possible that in this way the variations in endosinusal pressure caused by the cardiac cycle are minimized so far as impulses arriving at the vasomotor centre are concerned. Further, the large and small fibres probably have different characteristics of adaptation and summation, similar to the differences found for large tactile and small pain fibres in mixed sensory nerves (Adrian, 1932). If this is so, the reflexes subserved by the small barosensory fibres would probably be more effective in maintaining the arterial blood pressure at a constant level than those subserved by the large fibres.

In urethane overdosage, the thresholds for both pressor and depressor responses obtained by electrical stimulation of the sinus nerves rise at about equal rates and the character of the responses remains unchanged. In nembutal poisoning, however, the depressor reflexes suffer more than the pressor ones: when the anaesthesia is deep enough seriously to interfere with the activity of the respiratory centre but not so deep as to cause complete abolition of all vasomotor reflexes, then the depressor responses are no longer obtainable after stimulation of the sinus, aortic and vagus nerves while the pressor responses are still present. This phenomenon is reversible. On recovery from the overdosage with nembutal, either spontaneous or accelerated by picrotoxin, the normal depressor responses are again obtained. Proof has been given that the 'reversal' is not caused by a low arterial blood pressure and that centres higher than the vasomotor centres are unnecessary.

So far as the nature and the site of the 'reversal' are concerned, evidence is still incomplete. The following interpretation may serve as a tentative hypothesis. It is suggested that, while a strong stimulus activates chemosensory and both types of barosensory fibres in the sinus nerve in nembutal overdosage, the rise in blood pressure is brought about by the fact that the barosensory reflexes

are in abeyance. This is probably due to the barosensory reflex mechanisms of the medulla being more susceptible to nembutal than the chemosensory. This hypothesis could also explain why the vagal depressor response is converted to pressor in nembutal overdosage. However, the fact has to be taken into consideration that the fibre composition of the vagus is much more complex than that of the sinus nerve.

Of other anaesthetics, urethane has been found to have no selective effect on the barosensory mechanism. Neil, Redwood & Schweitzer (1948, 1949) have also recently described a 'reversal', apparently similar to that here described in nembutal overdosage, in cats anaesthetized with the normal dose of chloralose.

SUMMARY

1. Cats, anaesthetized with urethane or nembutal, had their lungs artificially ventilated after bilateral open pneumothorax. After section of both vagi and the left sinus nerve electrical stimulation of the right sinus nerve gave the following responses: (*a*) stimuli of low voltage and very short duration caused in many, but not in all, cats a fall in blood pressure; (*b*) when the voltage or the pulse duration was increased, a pressor response was obtained; (*c*) further increase, either in voltage or in pulse duration, caused at first an after-fall, then a biphasic and finally a depressor response. In responses (*b*) and (*c*), but not in (*a*), there was increased activity of the respiratory centre. It is suggested that response (*a*) is due to stimulation of large barosensory fibres with a low threshold, response (*b*) is due to smaller chemosensory fibres and response (*c*) is due to small barosensory fibres of high threshold.

2. Injection of half the original anaesthetic dose of nembutal in a cat, already anaesthetized with the normal dose of nembutal, resulted in a general fall in arterial blood pressure and in conversion of depressor responses, due to electrical stimulation of the sinus, vagus and aortic nerves, to pressor responses. No depressor response could be obtained while the cat was thus overdosed with nembutal, whatever the voltage or pulse duration of the stimulus. On spontaneous recovery from the effects of nembutal overdosage, or more rapidly after injection of picrotoxin, the normal depressor response reappeared. The depressor reflexes subserved by the vagus were less susceptible to the action of nembutal than those subserved by the sinus nerve.

3. Low arterial blood pressure by itself did not lead to a loss of the depressor reflexes.

4. Removal of the brain just cranial to the level of the entry of the 9th and 10th nerve in overnembutalized cats had no effect on either the pressor response or on the efficacy of picrotoxin.

5. Overdosage with urethane, unlike overdosage with nembutal, did not cause a selective loss of the depressor response. With increasing doses both pressor and depressor responses diminished and eventually disappeared.

6. Traction on the common carotid artery in the overnembutalized cat led to a pressor response which, although diminished, was still present after a cold block had been applied to the sinus nerve and which was similar to the pressor response obtained by traction on the cut sinus nerve.

7. Strong stimuli may activate the chemosensory and both types of barosensory fibres in the sinus nerve, yielding a depressor response in the cat under the normal anaesthetic dose of nembutal. The selective loss of the depressor responses found in over-nembutalized cats is assumed to be due to the barosensory reflex mechanism of the medulla being more susceptible to nembutal than the chemosensory. The vagal pressor response in nembutal overdosage can be similarly explained.

REFERENCES

Adrian, E. D. (1932). *Proc. Roy. Soc.* B, **109**, 1.
Blair, E. A. & Erlanger, J. (1933). *Amer. J. Physiol.* **106**, 524.
Bronk, D. W. (1931). *Proc. Soc. exp. Biol., N.Y.*, **28**, 1014.
Bronk, D. W. & Stella, G. (1932). *J. cell. comp. Physiol.* **1**, 113.
Douglas, W. W., Innes, I. R. & Kosterlitz, H. W. (1948). *J. Physiol.* **107**, 48*P*.
Douglas, W. W., Innes, I. R. & Kosterlitz, H. W. (1949). *J. Physiol.* **108**, 17*P*.
Euler, U. S., v. Liljestrand, G. & Zotterman, Y. (1939). *Skand. Arch. Physiol.* **83**, 132.
Euler, U. S., v. Liljestrand, G. & Zotterman, Y. (1941). *Acta physiol. Scand.* **2**, 1.
Gordon, G. (1943). *J. Physiol.* **102**, 95.
Heymans, C. & Rijlant, P. (1933). *C.R. Soc. Biol., Paris*, **113**, 69.
McDowall, R. J. S. (1925). *J. Physiol.* **59**, 41.
Neil, E., Redwood, C. R. M. & Schweitzer, A. (1948). *J. Physiol.* **107**, 8*P*.
Neil, E., Redwood, C. R. M. & Schweitzer, A. (1949). *J. Physiol.* **108**, 8*P*.
Walter, W. G. & Ritchie, A. E. (1945). *Electron. Eng.* **17**, 585.
Whitfield, I. C. (1946). *J. sci. Instrum.* **23**, 232.
Wright, S. (1932). *Brit. med. J.* i, 457.
Zotterman, Y. (1935). *Skand. Arch. Physiol.* **72**, 73.

J. Physiol. (1950) 111, 231–243 612.792.9.598.2

SOME HISTOCHEMICAL AND BIOCHEMICAL OBSERVATIONS ON THE PREEN GLAND

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In the course of histochemical examination, by one of us (D.B.C.), of the tissues of domestic fowls infected with Rous sarcoma, a high acid-optimum phosphatase activity was observed in the preen gland (*glandula uropygialis*). This observation, together with consideration of the distinctive oil secreting function of this gland, suggested that a study of the biochemistry and histochemistry of this organ might throw light upon problems of the metabolism of lipids.

A re-examination of the histology of the preen gland has therefore been carried out using modern histochemical staining methods. The tissue of the gland has also been examined for the presence of a number of enzymes, and certain of their characteristics determined.

The literature dealing with the anatomy, physiology and chemical nature of the secretion of the preen gland has been extensively reviewed by Hou (1928), who brought forward evidence that the secretion of the gland contained provitamin D (Hou, 1928, 1929, 1930). Our knowledge to-day might be briefly summarized as follows. The preen gland is present in most birds, but it is vestigial or absent in some birds of prey. It is a bilobed gland situated dorsally at the root of the tail feathers. Each lobe contains a small central cavity, which discharges an oily secretion through a muscular duct, which opens at the end of a nipple-like process on to the skin. Each lobe is composed of numerous glandular tubules arranged in a radial pattern. Each tubule has a central lumen containing fatty material surrounded by several layers of cells, which stain differently for fat in different parts of the tubule. It is clearly established that the secretion of the preen gland is spread by the bird over its plumage. Removal of the preen gland, with isolation of the bird, so that it cannot steal oil from normal birds, leads to ill health which can be relieved by adding vitamins of the D group to the diet.

Analyses of the secretion have revealed species differences. In general, however, it may be said that some 50% of the secretion is ether-soluble. The lipoid fraction contains both saponifiable and non-saponifiable fractions and lecithin; the non-lipoid constituents include protein and inorganic salts.

METHODS

The preen glands were excised immediately after the birds were killed. The glands were frozen on a glass plate cooled by solid CO_2, and 2 mm. thick slices were cut and fixed as described below for histochemical investigation. The rest of the tissue was kept at −20° C. until used for biochemical investigation. It was found that the secretion in the cavity of the lobe of the gland could be removed without difficulty by blunt dissection in the +4° C. refrigerator room.

Histochemical methods. For lipids, the method described by Dalton & Peters (1944) was used, involving fixation in Champy's solution for 24 hr., washing for 24 hr., and staining with 2% osmic acid at 38° C. for 5 days.

For alkaline phosphatase, Danielli's (1946) modification of the Gomori technique with fixation in 80% (v/v) ice-cold ethanol was used.

Acid phosphatase was demonstrated by the Gomori (1941) technique. The tissue was fixed in ice-cold absolute acetone for 24 hr. Sections were then incubated at 37° C. with an acetate buffer at pH 5·0 with α-glycerophosphate and $Pb(NO_3)_2$ for 2, 6 and 24 hr., and compared with controls incubated for similar times in a solution containing no α-glycerophosphate.

For polysaccharide, the periodic acid-Schiff (P.A.S.) technique of McManus (1948), with fixation in ice-cold 80% (v/v) ethanol for 24 hr., was used.

Biochemical methods. Ascorbic acid was estimated by grinding the tissue with a little sand and 15 ml. of 12% (w/v) trichloroacetic acid solution, filtering, and titrating the filtrate with 2:6-dichlorophenol indophenol. This dye was standardized against pure ascorbic acid.

For measurement of phosphatase activity, a tissue suspension was prepared by grinding the fresh tissue with twenty times its weight of 0·9% (w/v) NaCl solution and a little sand. 0·5 ml. of this suspension was added to 10 ml. of a freshly prepared 0·005 M solution of disodium phenyl phosphate in an appropriate buffer (usually Michaelis's barbiturate-acetate buffer). Samples were withdrawn for estimation of inorganic phosphate by the Bodansky (1932–3) method, immediately after setting up the digest and after incubation for 1 hr. at 37° C. The results are expressed in units defined as the number of mg. of inorganic P liberated under these conditions per 100 g. wet weight of tissue present in the digest.

Lipase and esterase activities were first measured by the method of Cherry & Crandall (1932), in which the substrate, emulsified with the aid of gum acacia, if not sufficiently soluble in water, and buffered at pH 7·0, is incubated with the enzyme preparation and the fatty acids liberated determined by titration with standard NaOH solution, using phenolphthalein as indicator. Later it was found that the manometric method of Rona & Lasnitzky (1924) was preferable, being more sensitive and giving a nearer approach to a linear relationship between the duration of the enzyme action and the quantity of substrate decomposed.

Preparations of preen gland tissue from the domestic hen were also examined for true and pseudocholine esterase by the method of Ammon (1934).

RESULTS

Histochemical findings

Anatomy. The general anatomy of the preen gland is shown in Pl. 1*a*—the preen gland of a 1-day-old chick. The two lobes of the gland are clearly shown, each with its central cavity containing an oily secretion which is discharged on to the surface of the skin at the tip of the nipple-like process by means of a duct

surrounded by smooth muscle fibres. The whole apparatus has been very aptly likened to an oil can.

Each lobe is made up of numerous tubules which radiate outwards from the central cavity to end blindly against the capsule of fibrous tissue enclosing each lobe. Trabeculae of fibrous tissue containing blood vessels, lymphatics and nerves separate the tubules. This fibrous tissue stains for alkaline phosphatase after incubation for 24 hr. This is a property which it has in common with fibrous tissue elsewhere and so no further mention of alkaline phosphatase need be made.

Demarcation of two zones in the preen gland by staining for acid phosphatase is shown in Pl. 1*b*, *c* and *d*, which show a sector of one lobe of the preen gland from a 25-day-old chick after incubation in the acid phosphatase substrate for 2, 6 and 24 hr. respectively. After 2 hr. incubation the secretion is jet black and some cells are showing granules staining for acid phosphatase; at 6 hr. the cells show considerable blackening, and by 24 hr. the staining is so intense that all histological detail is obliterated.

These photographs show the clear demarcation of two zones in the preen gland by the acid phosphatase staining technique—a central zone staining strongly positive and a peripheral zone in which the cells are unstained. Further study has shown that the central zone also contains cells rich in glycogen granules.

To have called this central zone the acid phosphatase zone would not have been strictly accurate, because the secretion stains positively in both zones. The central zone has therefore been termed by us the 'Glycogen Zone'. The cells of the outer zone contain no glycogen granules and do not stain for acid phosphatase, but have a close histological resemblance to the cells of the sebaceous glands of man. For clarity of description we have termed this outer zone the 'Sebaceous Zone'.

Description of a tubule in the glycogen zone. Pl. 1*e* shows the preen gland from a 20-week-old hen, after 6 hr. incubation in the acid phosphatase substrate. Both zones are shown, but it is only in the glycogen zone that the cells are staining for acid phosphatase. It will be noted that the secretion stains in both zones. When one tubule in the glycogen zone is examined under higher magnification (Pl. 1*f*) it will be noted that the cells which stain most strongly for acid phosphatase are those in the outer part of the tubule, lying near the supporting envelope of fibrous tissue. The staining is definitely granular in nature. This can be made out in Pl. 1*f*, but is more clearly demonstrated in Pl. 1*g*, which shows a higher magnification of the preen gland from the 25-day-old chick after 2 hr. incubation in the acid phosphatase substrate.

Glycogen granules. When the preen gland is stained by the periodic acid-Schiff (P.A.S.) technique (Pl. 2*a*) the glycogen zone is seen to contain cells with brilliant fuchsin-staining granules in their cytoplasm. These cells have the same position in the tubule as the cells which stain most strongly for acid

phosphatase. These P.A.S.-positive granules can no longer be demonstrated in sections which have been incubated in salivary amylase for ¼ or ½ hr., whilst control sections incubated in boiled saliva for 2 hr. still showed the granules. Additional evidence that these granules are glycogen is the fact that they stain well with Best's carmine stain (Pl. 2*b*). These glycogen-containing cells are not seen in the sebaceous zone.

Osmic acid staining. Prolonged staining with osmic acid also demonstrates differences between the glycogen and sebaceous zones. In the glycogen zone, the cells which border the lumen of the tubule contain numerous droplets of lipid which stain black with osmic acid. In the sebaceous zone it is the peripheral flattened cells lying against the connective tissue stroma which show lipoid droplets stainable with osmic acid (Pl. 2*c*, *d*).

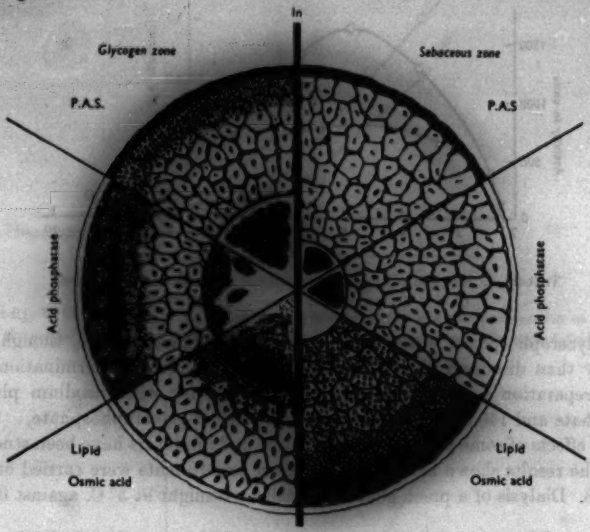
The cells of the glycogen zone stain more deeply with cytoplasmic stains than the cells of the sebaceous zone. Pl. 2*e* shows a section in which the tubules are cut longitudinally, and the sharp demarcation between the two zones is clearly demonstrated.

These appearances do not suggest that the sebaceous zone is forming the glycogen zone, or *vice versa*, by a process of differentiation because there is no intermediate transitional zone. In addition, as the cells in the tubules of both zones are differentiating from the periphery towards the central lumen there is no reason to suppose that there must be differentiation in a direction along the length of the tubule. The histochemical appearances would suggest a separate structure and function for the two zones.

Description of the tubule in the sebaceous zone. The outermost layer of cells, lying against the connective tissue stroma is flattened, stains well with cytoplasmic stains and contains droplets of lipid which stain black with osmic acid. Internal to these cells lie several layers of typical sebaceous cells. These are polygonal in shape, their cytoplasm comprising a foam of lipoid droplets which stain red with Scharlach R, but poorly with the osmic acid technique, which only outlines each droplet, giving a filigree pattern (Pl. 2*f*). The central sebaceous cells disintegrate to form the secretion. The lumen of the sebaceous tubule is much smaller than that of the glycogen zone tubule. The cells contain no cytoplasmic granules stainable by the acid phosphatase or P.A.S. techniques. Some sections, incubated for 24 hr. in the acid phosphatase substrate, may show a more or less universal staining of nuclear chromatin. In such sections the nuclei of the cells in the sebaceous zone are stained at 24 hr., but many preparations show no staining in the sebaceous zone at 24 hr., except for the secretion in the lumen of the tubules.

The secretion in both zones stains strongly for acid phosphatase even after only 2 hr. incubation. It also stains well with P.A.S. for polysaccharide, but this staining is unaffected by salivary amylase (Pl. 2*a*). Examination of the preen glands from two 3-year-old ducks showed an essentially similar structure.

The histochemical differences between the two zones as demonstrated by the three staining techniques described above are shown in diagrammatic form in Text-fig. 1.



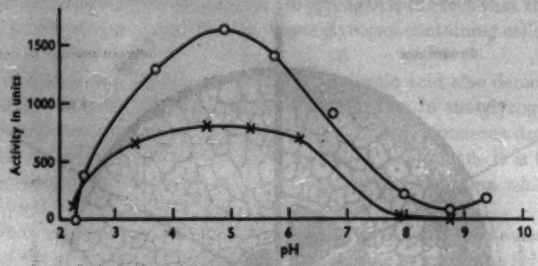
Text-fig. 1. Histochemical differences between the two zones of the gland. See text for further explanation.

Biochemical findings

Ascorbic acid content. The ascorbic acid content of thirteen normal preen glands from fowls was determined. The values observed varied from 16 to 63 mg./100 g. wet weight of tissue, with an average of 33·5 mg. In the course of other work similar observations were made on the preen glands of fifteen fowls bearing Rous sarcomata. The values observed varied from 7 to 35 mg./100 g. wet weight of tissue, with an average value of 22·2 mg.

Phosphatase activity. The phosphatase activity of fowl preen tissue homogenate was determined over a range of pH from 2·25 to 9·37 in barbiturate-acetate buffer. Determinations were made in duplicate with a maximum difference of 90 units in values ranging up to 1500 units. In a similar experiment, using a duck preen gland, duplicate differences did not exceed 14 units except at pH 3·3, where some difficulty was experienced in securing good reproducibility, and the value given for this pH is the mean of two observations

differing by 51 units, i.e. 9% of their mean. All pH values were checked with the glass electrode. The results obtained are shown in Text-fig. 2.



Text-fig. 2. Effect of pH upon the activity of preen gland phosphatase.
O —— O, fowl gland; × —— ×, duck gland.

β-Glycerophosphate is also hydrolysed by fowl preen tissue, though less readily than disodium phenyl phosphate. Thus duplicate determinations on one preparation gave 440 and 470 units of activity with disodium phenyl phosphate and 140 and 150 units of activity with β-glycerophosphate.

The effects of some phosphatase activators and inhibitors have been studied, with the results shown in Table 1. All these experiments were carried out at pH 4·8. Dialysis of a preen gland suspension overnight at 3° C. against 0·9%

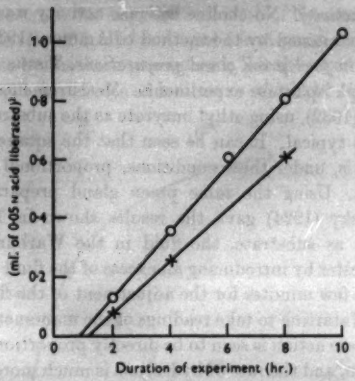
Table 1. Effects of some phosphatase activators and inhibitors upon the acid phosphatase of fowl preen gland. (Figures give the percentage activation (+) or inhibition (−))

Test substance	Concentration in digest		
	0·001 M	0·01 M	0·1 M
$MgCl_2$*	+13	+ 7	− 6
$MgCl_2$	− 2	− 8	−12
KCN	+ 3	± 0	+ 4
KF	−57	−77	−90

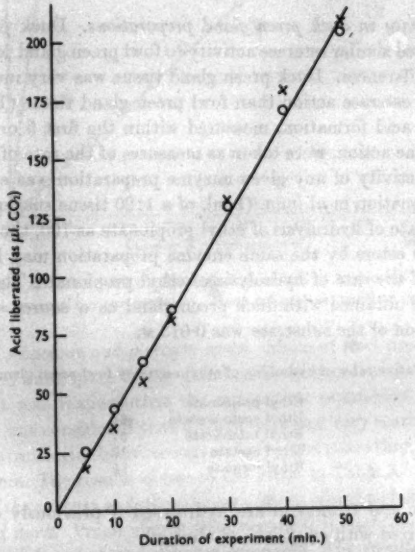
* The preen gland preparation used for this test had been dialysed for 16 hr. at 3° C. against 0·9% (w/v) NaCl solution.

(w/v) NaCl solution resulted in a loss of about 25% of its initial activity. It is considered that the small effects found on addition of Mg ions to these preparations are not sufficiently different from the possible results of experimental error to indicate either inhibition or activation. Up to 90% inhibition was obtained with KF, and no effect with KCN.

Solubility of the phosphatase. Centrifuging preen gland suspension in 0·9% (w/v) NaCl solution at 9000 r.p.m. for 30 min. left 32% of the original activity in an almost clear middle fraction. Solid material separated in layers at the top and bottom of the tube.



Text-fig. 3. Course of hydrolysis of ethyl butyrate by fowl preen gland suspension as measured by the method of Cherry & Crandall (see text). ○ and × indicate two independent experiments.



Text-fig. 4. Course of hydrolysis of ethyl butyrate by fowl preen gland suspension as measured by the method of Rona & Lasnitsky (see text). ○ and × indicate two independent experiments.

Choline esterase activity. No choline esterase activity was detected in preen gland suspensions examined by the method of Ammon (1934).

Esterase activity in fowl preen gland preparations. Tissue homogenates were prepared as in the phosphatase experiments. Measurements by the method of Cherry & Crandall (1932), using ethyl butyrate as the substrate, gave results of which Text-fig. 3 is typical. It can be seen that the square of the amount of the esterase action is, under these conditions, proportional to the duration of the enzyme action. Using the same preen gland preparation the method of Rona & Lasnitsky (1924) gave the results shown in Text-fig. 4. Ethyl butyrate was used as substrate, the fluid in the Warburg cup being kept saturated with the ester by introducing an excess of the fluid ester from the side arm and allowing a few minutes for the adjustment of the fluid volume before closing the taps and starting to take readings of the manometers. In Text-fig. 4 the amount of esterase action is seen to be directly proportional to the duration of the enzyme action, and the rate of hydrolysis is much more rapid than in the method of Cherry & Crandall. The Rona & Lasnitsky method was therefore used in all subsequent work. In general, substrate was supplied as 1 ml. of a 0·04 M solution of the ester in water, measured into the side arm of the Warburg cup.

Esterase activity in duck preen gland preparations. Duck preen gland preparations showed similar esterase activity to fowl preen gland preparations, but with certain differences. Duck preen gland tissue was very much more active with regard to esterase action than fowl preen gland tissue (Text-fig. 5). The initial rates of acid formation, measured within the first 5 or 10 min. of the period of enzyme action, were taken as measures of the rate of enzyme action, and thus the activity of any given enzyme preparation was expressed as the rate of CO_2 formation in μl./min./(1 ml. of a 1:20 tissue suspension).

Taking the rate of hydrolysis of ethyl propionate as 100, the rates of hydrolysis of various esters by the same enzyme preparation may be expressed as a percentage of the rate of hydrolysis of ethyl propionate. The results shown in Table 2 were obtained with duck preen gland as a source of enzyme, and the concentration of the substrate was 0·013 M.

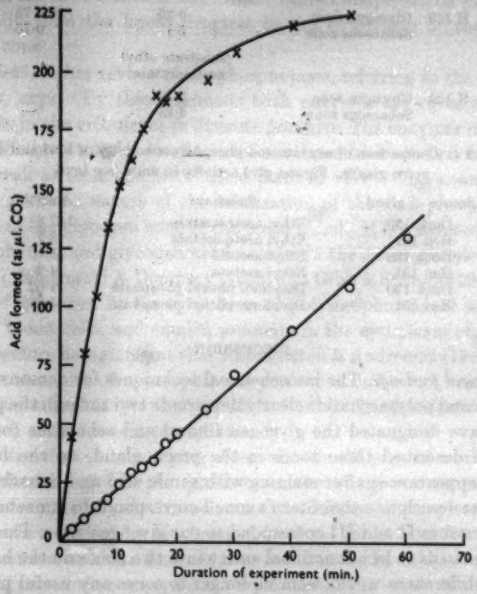
TABLE 2. Relative rates of hydrolysis of ethyl esters by fowl preen gland homogenate

Ethyl propionate	100
Ethyl aceto-acetate	64
Ethyl n-butyrate	37
Ethyl acetate	19
Ethyl formate	14

Ethyl laurate and tributyrin are hydrolysed if previously emulsified. No hydrolysis occurred with emulsions of olive oil.

A curious difference was found between fowl and duck preen gland suspensions with respect to the hydrolysis of ethyl butyrate present in excess in the

system. When this substrate was supplied as 0·5 ml. of the ester in the Warburg cup there was a linear rate of hydrolysis by the fowl preen gland tissue, but no measurable hydrolysis by the duck preen gland. Yet the same duck preen tissue suspension gave a rapid initial rate of hydrolysis of ethyl butyrate supplied as 0·013 M solution.



Text-fig. 5. Comparison of the rate of hydrolysis of ethyl acetate by fowl preen gland suspension, ○, and duck preen gland suspension, ×. Rona & Lasnitsky method (see text).

Comparison of sebaceous and glycogen zones. Slices of fowl preen gland were removed from the outer part of the gland and compared with the remaining inner portion. It was realized that the anatomical separation must be very imperfect, but it was considered that if there were any very marked differences in enzyme concentration in the sebaceous and glycogen zones then these analyses should reveal them. The results obtained are given in Table 3.

Comparison of the esterase and phosphatase activity of preen gland preparations from the fowl and duck. Preen glands were prepared in identical manners and their esterase and phosphatase activities measured. The results obtained are shown in Table 4.

TABLE 3. Comparison of enzyme activity of sebaceous and glycogen zones of fowl preen gland. Figures give activity in units (see text)

Preparation		Esterase activity (substrate ethyl butyrate)	Phosphatase activity at pH 4·8
H 131	Glycogen zone	8·3	1·30
	Sebaceous zone	8·5	1·28
H 129	Glycogen zone	6·25	0·75
	Sebaceous zone	5·1	0·75
		(substrate ethyl aceto-acetate)	
H 129	Glycogen zone	2·70	—
	Sebaceous zone	2·25	—

TABLE 4. Comparison of esterase and phosphatase activity of fowl and duck preen glands. Figures give activity in units (see text)

Source of gland	Substrate	Activity
Duck 130	Ethyl aceto-acetate	147
Hen 131	Ethyl aceto-acetate	8·7
Duck 130	Ethyl acetate	41
Hen 131	Ethyl acetate	4·2
Duck 130	Disodium phenyl phosphate	1·02
Hen 131	Disodium phenyl phosphate	1·30

DISCUSSION

Histochemical findings. The histochemical techniques for demonstrating acid phosphatase and polysaccharide clearly demarcate two zones in the preen gland, which we have designated the glycogen (inner) and sebaceous (outer) zones. Stern (1904) described three zones in the preen gland, on the basis of the histological appearances after staining with osmic acid and Scharlach R. It is clear from her description that Stern's zone I corresponds to our sebaceous zone, and that her zones II and III correspond to our glycogen zone. This division of what appears to us to be a functional entity into two zones on the basis of small anatomical differences would seem no longer to serve any useful purpose.

Lunghetti (1907) states that, between the 9th and 10th day of incubation in the hen, the epidermis forms two recesses, one on each side of the mid-line, which enlarge to form the central cavities of the two lobes of the gland. These primary buds give off thin epithelial pegs, secondary buds, from which the gland tubules are formed. This development in two stages is interesting in view of our demonstration of two zones with such different histochemical properties. Our own studies on the age at which glycogen, acid phosphatase and lipids appear in the developing preen gland are not yet complete and will have to form the subject of a separate communication.

As far as we are aware, it has not been suggested before that the different zones in the preen gland might be serving different functions. A reasonable interpretation of the histological and biochemical findings in the glycogen zone would be that the peripheral cells of the tubule, which contain glycogen

granules, and granules which stain for acid phosphatase, are differentiating into the cells bordering the lumen of the tubule, which are loaded with droplets of lipid. It is pertinent to inquire whether the glycogen is one of the raw materials out of which the lipid is being made, and what part the acid phosphatase is playing in the process. This lipid stains black with osmic acid very much more slowly than the subcutaneous fat. On the other hand, it stains with osmic acid more readily than the lipoid droplets in the cytoplasm of the cells of the sebaceous zone.

Moog (1946) in her review of the phosphatases, referring to the histochemical techniques, says: 'By these methods both enzymes are revealed in uniform distribution in the cell, never in discrete granules. The enzymes may, however, show specific intracellular localizations and have been studied from the cytological as well as from the histological point of view.' There can be no doubt about the granular nature of the localization of the acid phosphatase in the cytoplasm of the glycogen zone of the preen gland. It is quite possible that the acid phosphatase and glycogen are localized in the same granules.

Wislocki, Rheingold & Dempsey (1949) and Gibb & Stowell (1949) report the presence of glycogen in the cytoplasm of polymorphonuclear neutrophil and eosinophil blood cells, and smaller amounts in the cytoplasm of lymphocytes. It may therefore be significant that Rabinovitch & Andreucci (1949) report that the specific granules of neutrophil and eosinophil cells react strongly for acid phosphatase, and that lymphocytes show from two to five zones in their cytoplasm which stain for acid phosphatase.

Enzyme distribution. A striking observation in the present work has been the marked difference of acid phosphatase activity of the two zones of the preen gland as determined by the histochemical technique, in sharp contrast to their similarity when studied by biochemical methods on the homogenate. A tentative explanation of these findings may be that in the sebaceous part of the gland coenzyme and apoenzyme are separated in the intact tissue and remain separated during fixation and staining, so that no phosphatase activity is seen. In the glycogen zone they may be combined, and activity is demonstrable histochemically. The disintegration of tissue by grinding, in preparing the homogenate, would allow access of coenzyme to apoenzyme in tissue from the sebaceous zone. This suggestion would also explain the presence of an intensely staining secretion in the tubules of the sebaceous zone, where coenzyme and apoenzyme may combine after being excreted separately. Biochemical estimations of the acid phosphatase activity of the secretion have given comparatively low values, whilst the secretion stains intensely for acid phosphatase. It is possible that fixation of the tissue in acetone concentrates the phosphatase of the secretion by removal of the lipid.

Type of enzyme present. The phosphatase of the preen gland appears to be almost entirely of the type A_2 of Folley & Kay (1936). The discovery of this

phosphatase in very high concentration in the preen gland adds a new tissue to those in which acid phosphatase is already known to occur in high concentration, i.e. human prostate gland, spleen, liver, pancreas, kidney and erythrocytes. The human prostate is of course a very rich source, but the preen gland would appear to contain more acid phosphatase per unit weight than other readily available sources of this enzyme.

Esterase and lipase. It is of interest that true lipase, capable of hydrolysing the fat of olive oil, appears to be absent from fowl preen gland homogenate. This observation is in keeping with our finding that free fatty acid is absent from fresh preen gland secretion. So far as our investigations have gone, we can conclude that, in the case of the preen gland, the production of a secretion mainly composed of lipid is associated with the presence in the tissue of an esterase hydrolysing esters of short-chain fatty acids readily, and having no action on olive oil emulsion, and a phosphatase showing maximum activity at pH 4·8. This secretion of lipid proceeds without the presence in the tissue of demonstrable amounts of lipase acting upon the fat of olive oil, or of choline esterase.

The fact that glycogen can be demonstrated histochemically in the cells which have the greatest acid phosphatase activity may mean that this enzyme is associated with glycogen metabolism in the gland rather than with the metabolism of lipid. On the other hand, there is evidence to suggest that the glycogen may be a precursor of the lipid.

SUMMARY

1. The histology of the preen gland of the fowl and duck has been investigated by histochemical methods.

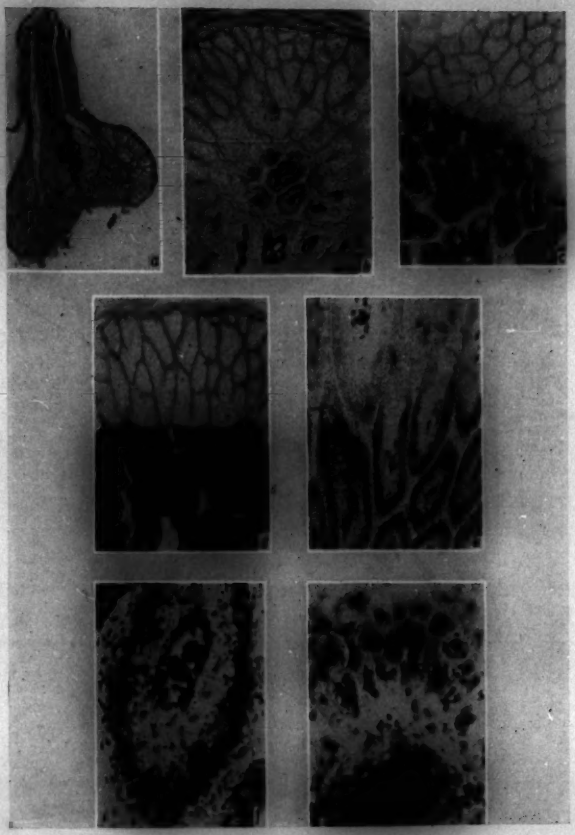
2. Evidence is presented for the existence of two zones in the gland, distinguished by histochemical staining reactions for acid phosphatase, glycogen and lipid.

3. The acid phosphatase of preen gland tissue has been shown to have a pH optimum of 4·8, to be insensitive to Mg ions and to KCN, but to be inhibited by KF.

4. An esterase present in preen gland tissue, which hydrolyses short-chain fatty acid esters, has been described.

5. The presence in preen gland homogenate of lipase and of choline esterase has been excluded.

We wish to express our gratitude to Prof. H. R. Dean for his advice and encouragement; to Dr P. E. H. Jones for testing the preen gland homogenate for choline esterase; to Miss J. Cook and Miss E. Douglas for technical assistance, and to Mr S. W. Patman for the photography.



(a)–(g)

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(a)–(f)

REFERENCES

Ammon, R. (1934). *Pflüg. Arch. ges. Physiol.* **233**, 486.
Bodansky, A. (1932–3). *J. biol. Chem.* **101**, 93.
Cherry, I. S. & Crandall, L. A. (1932). *Amer. J. Physiol.* **100**, 266.
Dalton, A. J. & Peters, V. B. (1944). *J. nat. Cancer Inst.* **5**, 99.
Danielli, J. F. (1946). *J. exp. Biol.* **22**, 110.
Folley, S. J. & Kay, H. D. (1936). *Ergebn. Enzymforsch.* **5**, 159.
Gibb, R. P. & Stowell, R. E. (1949). *Blood*, **4**, 569.
Gomori, G. (1941). *Arch. Path. Lab. Med.* **32**, 189.
Hou, H. C. (1928). *Chin. J. Physiol.* **2**, 345.
Hou, H. C. (1929). *Chin. J. Physiol.* **3**, 171.
Hou, H. C. (1930). *Chin. J. Physiol.* **4**, 79.
Lunghetti, B. (1907). *Arch. mikr. Anat.* **69**, 264.
McManus, J. F. A. (1948). *Stain Tech.* **23**, 99.
Moog, F. (1946). *Biol. Rev.* **21**, 41.
Rabinovitch, M. & Andreucci, D. (1949). *Blood*, **4**, 580.
Rona, P. & Lasnitzky, A. (1924). *Biochem. Z.* **152**, 504.
Stern, M. (1904). *Verh. Ges. dtsch. Naturf. Ärzt.* **76**, 419.
Wislocki, G. B., Rheingold, J. J. & Dempsey, E. W. (1949). *Blood*, **4**, 562.

EXPLANATION OF PLATES

Plate 1

(*a*) Preen gland of a day-old chick. ×20. Azan.

(*b*) Preen gland of a 25-day-old chick. Acid phosphatase. 2 hr. incubation. 16 mm. ×6.

(*c*) Preen gland of a 25-day-old chick. Acid phosphatase. 6 hr. incubation. 16 mm. ×6.

(*d*) Preen gland of a 25-day-old chick. Acid phosphatase. 24 hr. incubation. 16 mm. ×6.

(*e*) Preen gland of hen aged 20 weeks. Acid phosphatase. 6 hr. incubation. 16 mm. ×10.

(*f*) Higher power of tubule in glycogen zone from (*e*). Acid phosphatase. 6 hr. incubation. 4 mm. ×8.

(*g*) Tubule from glycogen zone of 25-day-old chick. Acid phosphatase. 2 hr. incubation. Shows the granules in the cells. 2 mm. ×8.

Plate 2

(*a*) Tubule in glycogen zone stained with P.A.S. to show glycogen in the cells. 4 mm. ×8.

(*b*) The margins of three tubules in the glycogen zone stained with Best's carmine to show glycogen. 4 mm. ×8.

(*c*) Preen gland, osmic acid and neutral red. Shows lipid in the glycogen zone. 16 mm. ×10.

(*d*) High power of (*c*) showing lipid near the lumen of tubule in glycogen zone. 4 mm. ×8.

(*e*) Tubules cut longitudinally showing the sudden change from sebaceous zone (top) to glycogen zone (bottom). Osmic acid, neutral red. 16 mm. ×10.

(*f*) Tubule in sebaceous zone showing 'filigree' pattern in sebaceous cells and the peripheral cells stained with osmic acid. 4 mm. ×8.

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A CYTOPLASMIC CONSTITUENT OF BRAIN

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Morrison & Hack (1949) have found that the histiocytes of the spleen in Gaucher's disease give a rose-purple colour when stained by the periodic acid-leucofuchsine method. They suggested that this reaction is due to the presence of cerebrosides in the Gaucher cells and they showed that cerebrosides do, in fact, give this reaction.

Since nervous tissue is known to be rich in cerebrosides, it was decided to study the reaction of the cells of the central nervous system to the periodic acid-leucofuchsine technique. Morrison & Hack found the Gaucher substance to be preserved in paraffin sections and even to be resistant to hot ethanol, whereas it is to be expected that lipids of myelin would be removed during the process of embedding in paraffin. It was therefore considered that application of this staining technique to sections of brain and spinal cord embedded in paraffin might give useful information on the location of glycolipids other than those present in myelin. Lillie (1948) noted that the blood vessels of the brain react well with periodic acid-leucofuchsine, and McManus (1948) stated that myelin stains in frozen sections. Cater (1949), using this stain, observed a pink colour in sympathetic ganglion cells near the adrenal gland, but the authors are not aware of any account of a reaction by neurones of the central nervous system.

METHODS

Brain tissue of rabbits killed by a blow on the neck followed by bleeding was used. Small pieces of tissue were cut from the fresh brain with a mounted razor blade. In each case some tissue was fixed directly by immersion in 10% formol saline for 2 days and then dehydrated and embedded in paraffin wax, and sectioned.

In the first case (rabbit no. 1) pieces of cerebrum, cerebellum and cervical cord from the same rabbit were fixed and embedded and sectioned in the above manner.

In the second case (rabbit no. 2) pieces of cerebrum and cerebellum were treated in two ways. Some tissue was fixed with formol saline and embedded in the usual manner. Other pieces of cerebral and cerebellar tissue were placed directly into pyrex flasks containing 20–40 ml. ethanol-ether mixture (3 vol. ethanol, 1 vol. diethyl ether). The flasks were fitted by ground glass joints to reflux condensers. They were boiled on a hot plate with reflux condensation for 2 hr. The supernatant fluid was then discarded and the extraction of the tissue under reflux was repeated with fresh ethanol-ether mixture. After this combined process of fixation, extraction of lipids and dehydration, the tissues were transferred to xylene, embedded in paraffin wax, and sectioned.

In the third case (rabbit no. 3) three pieces of cerebral cortex (parietal region) were taken from

the same rabbit. Two pieces (symmetrical portions from left and right hemispheres) were placed directly in formol saline for 2 days. One of these was then dehydrated and embedded in paraffin wax, while the other was first extracted with ethanol-ether mixture (40 ml.) under reflux condensation for 2 hr. and then with fresh solvent for a further 4 hr. This latter piece of cerebral cortex was then transferred to xylene and embedded in paraffin wax. The third piece of cerebrum from this same rabbit was fixed by direct extraction with two changes of ethanol-ether mixture (40 ml. each time) and then transferred to xylene and embedded, thus following the method used with tissue from rabbit no. 2.

The periodic acid-leucofuchsine technique of McManus (1948) (see also Hotchkiss, 1948) was used for staining the sections. 0·5% aqueous periodic acid (B.D.H.) was applied for 5 min. After washing with distilled water Schiff's reagent was applied for 30 min. Otherwise the method of McManus was followed except that counter-staining with haemalum was omitted since this might obscure histochemical results.

Toluidine blue (1% aqueous solution) was employed as a stain for Nissl substance in other sections. This stain was applied for 1 min. and then differentiated by passing through 90–95% ethanol and then to absolute ethanol and xylene.

RESULTS

The cell bodies of many of the neurones of the cerebellum, cerebrum and the spinal cord were brightly and distinctively stained and showed a vivid rose-pink colour. The only other structures in these tissues which gave an intense reaction were the walls of blood vessels.

Cerebellum (Pl. 1*a*, *b*). The row of rose-pink Purkinje cells at the junction of the molecular and granular layers of the cortex was a most conspicuous feature of the cerebellar sections. The granule cells were for the most part only faintly stained, and the molecular layer formed a faint pink background. In some sections the aborizing dendrites of the Purkinje cells coursing up through the molecular layer were also well shown, since they stained a deeper pink than the background. By careful inspection it was possible to discern that the apparently diffuse pink colour in many of the cells was present in extremely fine cytoplasmic granules. The nuclei of the Purkinje cells appeared to be unstained, but the nucleoli had a definite pink colour. Control sections without periodic acid gave hardly any reaction.

Cerebrum (Pl. 1*c*). The cell bodies of many of the neurones of the cerebral cortex also gave a bright rose colour. Groups of reacting pyramidal cells, as well as the blood vessels, showed rose pink on a pale pinkish background. The pink colour appeared in part, at any rate, to be located in very fine rose granules in the neurones. Where nuclei could be distinguished they appeared unstained and nucleoli were pink. The neurones in control sections without periodic acid were practically colourless.

In the cerebral cortex, however, numerous other neurones including pyramidal cells did not give this bright reaction to periodic acid-leucofuchsine. This differentiation into two classes of cells was also apparent in sections stained with toluidine blue. The cells which reacted vividly to both stains appear to correspond to the chromophilic cells which have recently been

studied in the cerebral cortex of the cat by Miller (1949). Miller, using various dyes including toluidine blue, has distinguished the more faintly reacting neurones with large nuclei from the groups of deeply staining chromophilic cells with smaller nuclei. It is apparently these latter cells which react so intensely to periodic acid-leucofuchsine.

Spinal cord. The large motor neurones of the anterior horns of the cervical cord gave a definite pink colour, though not such an intense reaction was observed here as with the neurones of the brain. The nuclei appeared as empty unstained spaces. A control section without periodic acid gave hardly any reaction.

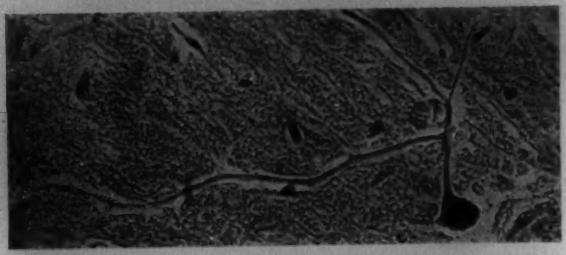
Nature of the chromogenic material. The rose-pink staining material has a general location in the nerve cell similar to that of the Nissl substance stained by toluidine blue. Both are situated in the cytoplasm of the cell body and in the dendrites. The substance reacting to periodic acid-leucofuchsine is in part, at any rate, present in fine granules.

In tissue fixed initially by extraction under reflux condensation with boiling ethanol-ether mixture (rabbits nos. 2 and 3, see Methods) the neurones showed little of the specific reaction. By this procedure of extraction the distinctive rose-pink coloration of the vast majority of the Purkinje cells of the cerebellum was obliterated, leaving pale pink cells hardly differing from the ground substance in colour. The same treatment also eliminated most of the distinctive rose-pink reaction of the pyramidal cells of the cerebral cortex. Sections from tissue extracted in this way, however, still showed considerable staining of the cell bodies by toluidine blue. Even when fixation in formaline preceded extraction (as used with one block from rabbit no. 3) the specific reaction was again considerably diminished, though not to such a great extent as when ethanol-ether mixture was the sole fixative. Here again much of the staining of the neurones by toluidine blue persisted after the extraction which had so much diminished the reaction to periodic acid-leucofuchsine.

After fixation and extraction with boiling ethanol-ether mixture, the elastic laminae of the arterioles and the basement membranes of the capillaries for the most part still stained sharply with periodic acid-leucofuchsine. Thus the substance in the neurones which is removed by the extraction would appear not to be the same as the glycoprotein which is present in the walls of the vessels. Furthermore, it seems unlikely that a glycoprotein would be removed by such extraction.

Incubation with saliva for 1 hr. at 37° C. failed to remove the substance reacting to periodic acid-leucofuchsine. Just as pronounced a reaction was given after incubation with saliva under conditions where glycogen was completely removed from control sections of liver. Thus the neuronal chromogenic substance cannot be glycogen. It would seem also to be quite distinct from the Nissl-staining material which Szent-Györgyi (1931) claimed to have removed from neurones by treatment with saliva.

(a)



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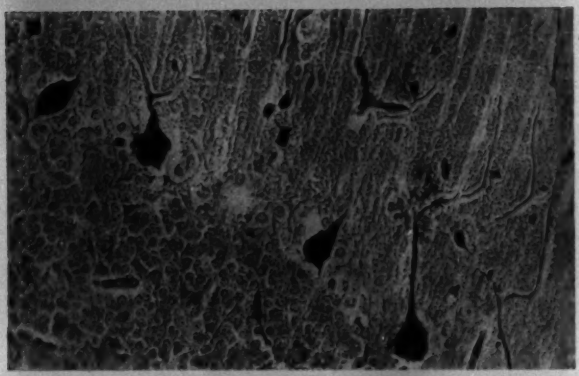

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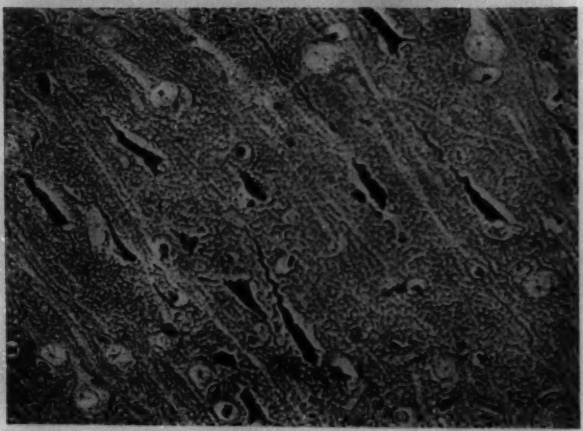

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DISCUSSION

From the above results it is suggested that the chromogenic substance in neuronal cytoplasm is a glycolipid. It is removable by prolonged extraction with ethanol-ether mixture. It appears to be discrete from the cytoplasmic nucleoprotein of the Nissl substance, although it may be in close proximity to the tigroid bodies. In any case, pentose nucleic acid does not contain vicinal hydroxyl groups and so does not react with periodic acid-leucofuchsine (Hotchkiss, 1948). Fat-soluble substances containing galactose, and thus having vicinal hydroxyl groups in their configuration, include both the cerebrosides and the gangliosides. The former appear to be one of the main constituents of myelin (Johnson, McNabb & Rossiter, 1948), and thus predominate in white matter and nerve. The latter, however, are apparently mainly confined to grey matter (Brante, 1949; Thannhauser & Schmidt, 1946; Klenk & Langebeins, 1941) and so would appear more likely to be the glycolipids responsible for this reaction of the cells of the brain to periodic acid-leucofuchsine.

SUMMARY

1. A substance is present in the cell bodies of many of the neurones of the central nervous system which reacts vividly to the periodic acid-leucofuchsine stain.

2. Extraction of the fresh tissue with boiling ethanol-ether mixture greatly diminishes the intensity of the reaction.

3. The reaction is not diminished by digestion with saliva.

We wish to thank Prof. H. R. Dean for his kind interest in this work. We are very grateful to Mr D. Madin for his valuable technical assistance throughout and to Mr S. W. Patman for the microphotography.

REFERENCES

Brante, G. (1949). *Acta physiol. Scand.* **18**, Suppl. 63, p. 172.
Cater, D. B. (1949). Personal communication.
Hotchkiss, R. D. (1948). *Arch. Biochem.* **16**, 131.
Johnson, A. C., McNabb, A. R. & Rossiter, R. J. (1948). *Biochem. J.* **43**, 573.
Klenk, E. & Langebeins, H. (1941). *Hoppe-Seyl. Z.* **270**, 185.
Lillie, R. D. (1948). *Histopathologic Technic*, p. 188. Philadelphia and Toronto: The Blakiston Co.
McManus, J. F. A. (1948). *Stain Tech.* **23**, 99.
Miller, R. A. (1949). *Amer. J. Anat.* **84**, 201.
Morrison, R. W. & Hack, M. H. (1949). *Amer. J. Path.* **25**, 597.
Szent-Györgyi, A. (1931). *Nature, Lond.*, **128**, 761.
Thannhauser, S. J. & Schmidt, G. (1946). *Physiol. Rev.* **26**, 275.

EXPLANATION OF PLATE

a, *b*, *c*: microphotographs of sections of brain (rabbit) stained by periodic acid and Schiff's reagent, ×320. *a*, Purkinje cell from cerebellar cortex with branching dendrites. *b*, row of Purkinje cells at the junction of molecular and granular layers of cerebellar cortex. The cell bodies and their antler-like branching dendrites are conspicuous. Some small cells of the molecular layer are also deeply stained. *c*, pyramidal cells from cerebral cortex with their spiral apical dendrites, both giving a strong reaction.

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ACTION POTENTIALS FROM A SENSORY NERVE ENDING

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The initiation of sensory impulses presents a series of problems: (*a*) the transmission of the stimulus from its external source to the receptor cell; (*b*) the mechanism whereby the stimulus is transformed into an electrical membrane change capable of exciting nerve terminals; (*c*) the initiation of impulses at the nerve ending and their propagation into the afferent axon trunk. An attempt has been made here to throw further light on these problems by studying the electrical changes in a sensory nerve fibre at a point close to its peripheral terminals. It is clear that only the last aspect of the problem (*c*) is directly amenable to electrical investigation, but such a study may also provide important clues to the preceding links of the process (*a* and *b*).

A suitable preparation was obtained from the muscle spindle of the frog. The electric response in the sensory axon close to the spindle differs in certain respects from a simple nerve spike, and some of these features can be related to the local events which take place in the sense organ. In the present paper the action potential in the terminal portions of the fibre will be described. It will also be shown that the initiation of an impulse at a sensory nerve ending does not invariably lead to a propagated message in the main axon. In the following paper, stretching of the muscle will be shown to depolarize the sensory nerve endings and thereby give rise to a repetitive discharge of impulses.

METHOD

Preparation. The experiments in this and the following paper were made on stretch receptors of the M. extensor longus dig. IV of the frog, the results being obtained from seventy-nine preparations at temperatures of 15–24° C. The muscle is supplied by a branch of N. peroneus. Before entering the muscle the nerve divides into a few twigs of which the proximal one often contains a motor and two sensory axons. Occasionally, one or two sensory fibres leave the N. peron. lat. several millimetres above the point of departure of the main branch to the muscle and, surrounded by a separate connective tissue sheath, run to the proximal part of the muscle. After the muscle with its nerve had been isolated, one of the proximal axons was selected, and all other nerve fibres were cut. The remaining axon could often be seen to end within a millimetre of its point of entry into the muscle, and many of these preparations were mounted without further dissection. Some muscles were divided until the intramuscular part of the sensory fibre had been cleared and the

spindle with a portion of the intrafusal muscle bundle freed from adjacent tissue (cf. Fig. 1). In such cases the recording electrode could be placed a little closer to the nerve endings, but the results were substantially the same with either type of preparation.

The intramuscular course of the nerve and the position of the sensory endings was checked after many experiments by staining the preparation with osmic acid. The histological pattern of the sensory terminations varies a good deal. In some cases the axon is connected to a single end organ and

Fig. 1. Photomicrographs of frog muscle spindles. A, isolated living spindle, immersed in Ringer solution, showing capsule, intrafusal muscle fibres and nerve supply. The nerve contained a sensory and a motor axon which was cut. B, stained preparation (osmic acid). Muscle with two sensory axons one of which was cut. The muscle was flattened between slides before fixation.

does not divide into branches until it has reached, or entered, the spindle capsule. In other cases, the sensory nerve fibre enters the muscle and then divides into two branches which supply separate spindles (cf. Young, 1950). These end organs are located either on the same or on different intrafusal muscle bundles. Finally, the axon may divide before entering the muscle. The results described in this paper were obtained in preparations in which the fibre divided *distal to*, and not *between*, the recording leads. This is important because complications arise if a bifurcation of the axon occurs between the recording electrodes; in this case an afferent impulse starting from one of the spindles passes the distal lead and returns to it by 'axon reflex', and this alters the shape of the spike potential.

Apparatus. The recording system has been described elsewhere (Katz, 1949). The recording electrodes consisted of chlorided silver wires connected to the preparation through capillary tubes filled with Agar-Ringer. The amplifier was either direct coupled or had coupling condensers causing its response to decline to one-half in about 0·25 sec. In several experiments a condenser and grid leak were placed into the input lead to check whether the grid current of the first valve (about 10^{-12} A.) affected the nerve fibre. For antidromic stimulation, platinum electrodes were applied to the sciatic nerve, and brief thyratron shocks were used, isolated from earth by means of an air-cored transformer.

The tendons of the muscle were clamped by ruling pens and the whole preparation lifted into oil. Two recording electrodes were applied, usuall one to nerve and one to muscle (Fig. 2). There are obvious advantages in placing the electrodes on relatively robust and immobile parts of the preparation, and therefore, when the whole muscle was used, the recording leads were applied preferably to the end of the muscle and to the peroneal nerve, rather than to the isolated portion of the axon. The muscle and nerve tissues on either side of the isolated stretch merely acted as low-resistance connectors without contributing any noticeable electric response themselves. This was verified by short-circuiting the sensory axon (letting it lie along the muscle) which made the afferent response invisible, and by moving the recording electrodes along muscle or nerve trunk, there being no noticeable change in the shape of the afferent spike until the electrodes were moved on to the isolated axon itself. However, the present method could not be used for recording *antidromic* impulses in the sensory fibre: in this case the central electrode had to be applied to the isolated axon itself, to avoid recording responses of cut fibres of the peroneal nerve.

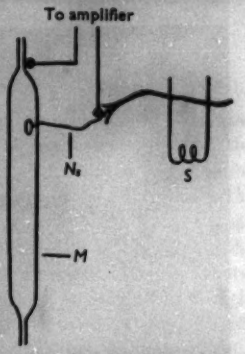

Fig. 2. Electrode arrangement showing stimulating (*S*) and recording leads. *M*, M. extensor longus dig. IV; N_s, isolated sensory fibre.

The isolated axon, together with its sheath, was usually 30–60 μ. thick and a few millimetres long. The resistance of this stretch was very high, and in some experiments considerably magnified the time constant of the input circuit. Hence, the spike potential was sometimes appreciably slowed and attenuated. Square pulse calibrations indicated that the time constant of the delay of the recording system varied in different preparations between less than 40 and 250 μsec. In the former case, the distortion of the action potential was slight, but in the latter case the peak of the spike may have been reduced by as much as 50%. The size of the spike (usually 2–3 mV.) was, however, of little interest, and the interpretation of the records was not seriously affected by this amplifier lag.

To summarize the present technique, electric responses are led off from a sensory axon at two points, a few millimetres apart. The distal lead is close to the sensory terminals of the spindle, but not in direct contact with them. Even when the spindle had been isolated (Fig. 1) the terminal structures remained enclosed inside the spindle capsule, and any localized potential changes could only be recorded at a distance determined by the length of the intra-capsular nerve branches. The electrotonic spread along the fine terminal branches is bound to involve an appreciable decrement, and the residual signal recorded on the outside of the capsule must be a reduced and distorted image of the local events at the sensory endings. It is important to keep this reservation in mind when interpreting the potential changes recorded with the present method.

RESULTS

When the tension of the resting muscle is low, afferent impulses are discharged occasionally, at apparently irregular intervals. If these impulses are recorded in the neighbourhood of the spindle, two characteristic phenomena are observed which differ from the properties of the ordinary conducted axon spike:

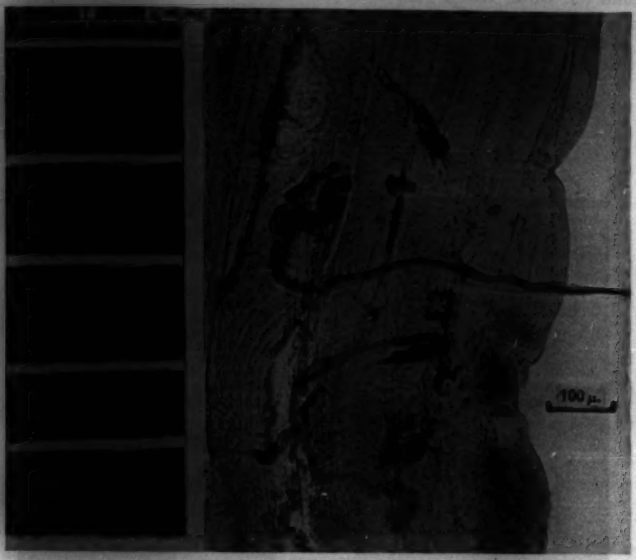

Fig. 3. (Left): Afferent impulse from a muscle spindle. Note: All records, in this and the subsequent paper, read from left to right. Downward deflexion means 'spindle negative' (i.e. a positive potential difference between the proximal and distal recording electrode). The spikes were recorded on a fast repetitive time-base, without synchronization. (Right): Photomicrograph of same muscle with single sensory axon and spindle. The preparation was treated as in Fig. 1 B.

(i) instead of a diphasic wave, a triphasic potential change is seen the third phase of which will be shown to be a residual depolarization of the nerve endings; (ii) the afferent impulses are initiated by discrete local action potentials which either remain abortive or lead to a propagated spike after a small but visible delay. The first observation suggests that the terminal portions of the nerve fibre differ in certain respects from the axon trunk; the second phenomenon indicates that there are discrete regions of low safety margin, or 'partial block' in the initial path of the afferent impulse.

A localized 'negative after-potential'

Figs. 3–5 illustrate the shape of typical afferent spikes recorded with the distal lead close to, i.e. within less than 1 mm. of, the spindle capsule. The triphasic potential change was observed in every fresh preparation, provided the sensory axon was not injured nearby or partly depolarized by preceding stimuli (cf. Katz, 1950). The third phase lasts several milliseconds at 20° C. and may reach 40–50% of the initial spike deflexion.

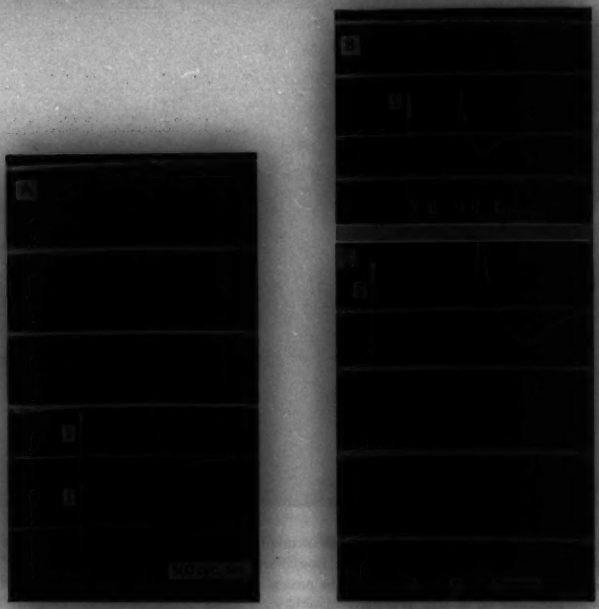

Fig. 4. Afferent (first deflexion downward) and antidromic (first deflexion upward) impulses in single sensory axons. In experiment A, the second record from below shows a superposition, by chance, of an antidromic and afferent impulse. *S*, stimulus artifact preceding the antidromic impulse.

When the proximal lead was applied to an injured region, the afferent impulse became monophasic and showed a prolonged tail portion which corresponded to the third phase previously observed. It appears that the electric response at the terminal portions of the axon is more prolonged than along the rest of the fibre and thereby gives rise to a third phase. Such an effect may be described as a 'terminal negative after-potential'.

A corollary to this phenomenon was observed when afferent and antidromic spikes were compared (Figs. 4, 5). This was done by stimulating the sciatic nerve with a short shock, without moving the recording electrodes. There are

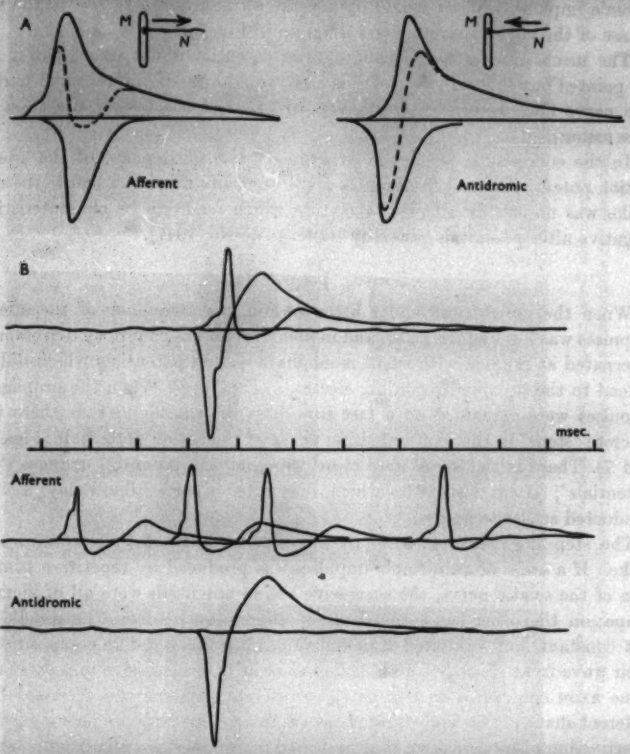

Fig. 5. A, diagram illustrating the assumed composite nature of the afferent and antidromic spike record. In this figure 'spindle negativity' (first deflexion of afferent spike) is shown upward. *M*, muscle; *N*, nerve. B, tracings of afferent and antidromic spikes. Same experiment as in Fig. 4, B and C.

obvious differences in the shapes of the two action potentials, the afferent spike being triphasic, with a brief and small second phase, while the antidromic impulse is diphasic with a large and prolonged second phase. When the two action potentials are superimposed, as in Figs. 4 and 5, their final phases are seen to be identical in time course and electrical sign, although the initial spikes

have opposite polarity. These observations can be explained in the following way. If there is a prolonged depolarization at the terminal portions of the fibre, this would add its effect to the second phase of the descending antidromic impulse, while it would oppose the second, and give rise to the third phase of the afferent impulse (see diagram in Fig. 5).

The mechanism of this localized after-potential is not clear, but it may be pointed out that an effect of this kind would arise if the terminal parts of the nerve fibre were more strongly polarized than the rest of the axon (see Discussion).

In the course of a prolonged experiment, the third phase of the afferent action potential tended to diminish and eventually to vanish before the main spike was noticeably affected, a feature which seems to be characteristic of negative after-potentials generally (Lorente de Nó, 1947).

Sensory 'pre-potentials'

When the muscle was under little tension, the frequency of the afferent impulses was low and irregular, and in many preparations propagated impulses alternated at random with small monophasic action potentials which did not spread to the proximal recording electrode (Figs. 6–8). When the propagated impulses were examined on a fast time base, it was found that there were discrete 'steps' in the foot and rising phase of the action potentials (Figs. 3–5 and 7). These initial steps were clearly preliminary potential changes ('pre-potentials', Arvanitaki, 1939) which, only after a perceptible delay, led to a conducted sensory impulse.

The step-like rise was shown by the afferent, but not by the antidromic, spike. If a series of antidromic impulses was produced by repetitive stimulation of the sciatic nerve, the successive action potentials were all of identical shape; on the other hand, the shape of afferent spikes from the spindle was not constant, but exhibited a definite 'play' in the step-like composition of their wave front (Figs. 3–5 and 7). It was clear that successive impulses in the same axon conformed to two or three discrete patterns characterized by a different shape of foot and rising phase. Similarly, in those preparations where abortive impulses were seen, they appeared in two or three recurrent sizes. In the experiment of Fig. 6B, for instance, random responses from an almost slack spindle were recorded during a period of approximately 5 sec. Out of 74 detectable action potentials, 30 were propagated (relative size: 100), while the rest were abortive impulses of two, or possibly three discrete amplitudes (relative sizes: 25, 12 and 8–10; frequencies: 14, 1 and 29, respectively). In another experiment, during a period of 8·5 sec., 112 action potentials were recorded of which 70 were propagated (size: 100), while the others were local responses (sizes: 32, 10 and 5; frequencies: 7, 18 and 17, respectively).

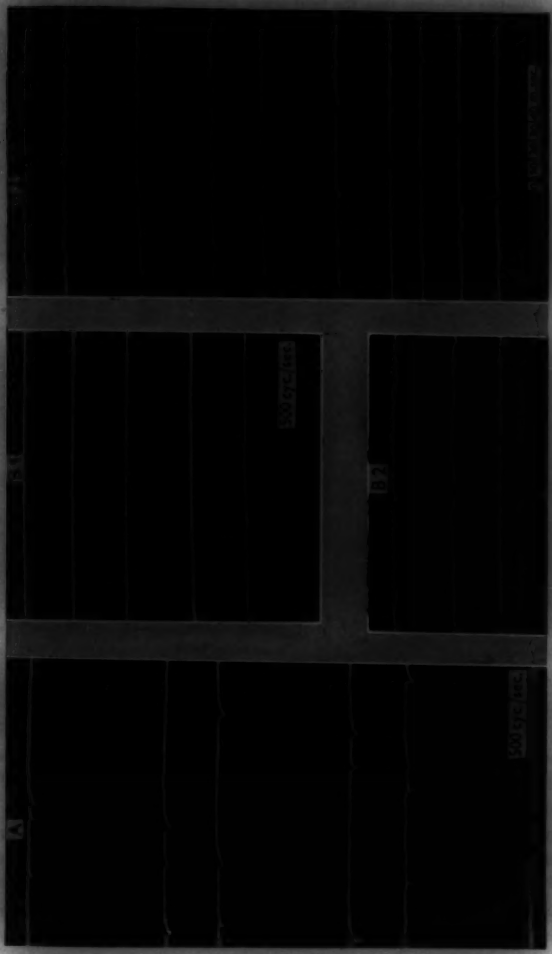

Fig. 6. Afferent discharges in single axons, showing occasional abortive spikes (small and monophasic). A and B2, single sweep records; B1, multiple sweeps superimposed. 'Spindle-negativity' downward.

It should be noted that the propagated spikes are likely to have been attenuated more severely by the present recording technique than the local action potentials (see Method), and that the relative size of the local responses given by the above figures may be somewhat too high.

Another significant fact was found when the intervals between successive afferent potential changes were measured. In the almost slack preparation, these intervals varied over a wide range, but it was clear that after a *propagated* impulse the preparation became silent for at least $\frac{1}{10}$ sec., that is no spike, full-size or abortive, could be observed during this time. It was equally clear that after an *abortive* impulse no such silent period occurred. For example, in the second experiment quoted above, the least observed interval between a propagated spike and the next, local or propagated, action potential was 50 msec. (the maximum being 290 msec.), but the shortest interval between an abortive impulse and the next potential change, local or propagated, was about 1 msec.

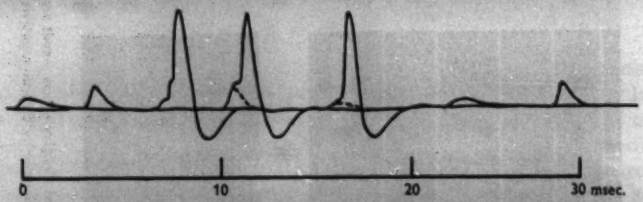

Fig. 7. Tracings of propagated and abortive spikes in a sensory axon. 'Spindle negativity' upward. Same experiment as in Fig. 6 B.

Abortive impulses were observed when the muscle was almost slack and its afferent discharge infrequent and irregular, but they disappeared on stretching and gave way to a regular series of propagated spikes (Fig. 8). They also disappeared, together with all afferent response, when the spindle was subjected to a bombardment by antidromic impulses at a rate of about 100 per sec. The interval between the antidromic spikes at this frequency was longer than the refractory period of the nerve, but shorter than the repetition period of the afferent discharges in the almost slack muscle. The stoppage of all afferent potentials indicates that the antidromic impulses must have reached the active terminals of the spindle and made them temporarily irresponsive (see also Matthews, 1931). Thus, while the afferent potentials were not all propagated, the antidromic impulses never seemed to fail in reaching the terminal structures.

The properties of the 'pre-potentials' here described differ in important respects from the local responses found in nerve during subthreshold stimulation, or at the end-plate region during neuromuscular block. The sensory pre-potentials occur in discrete quantal sizes and are not subject to continuous gradation which is quite different from the end-plate potential or the local cathodic response in nerve. This observation provides an interesting clue,

and it suggests that we are dealing with discrete sensory spikes which have started in one of the terminal branches, but for some reason encounter an obstacle before propagating into the axon trunk. It appears that the spindle contains a number of terminal units which—at least under a condition of low tension—are capable of firing separately and producing miniature spikes. Such

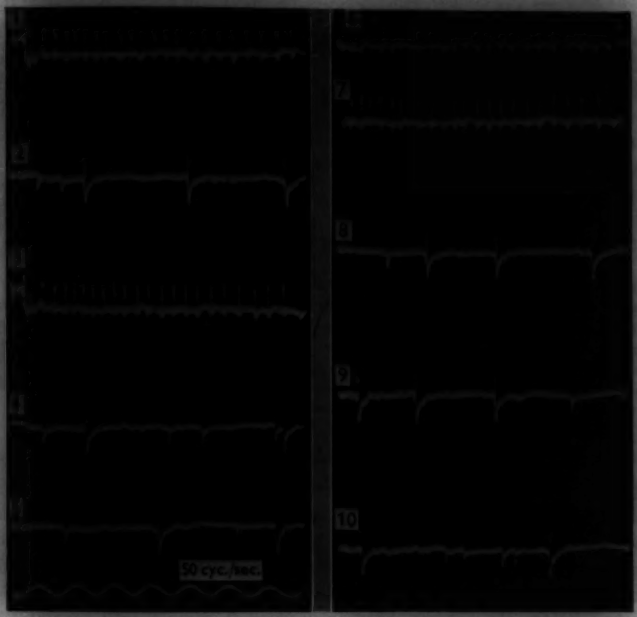

Fig. 8. Effect of moderate stretching on propagated and abortive spikes. In records 2, 4, 5 and 8–10, the muscle was under little tension, 14–15 mm. long. Note occasional abortive spikes. In records 1, 3, 6 and 7 the muscle was stretched to a 1·2 mm. greater length. Note burst of propagated discharges, but no sign of abortive spikes.

a miniature spike might raise the excitability of other near-by terminals by means of its electrotonic spread, and it apparently can sum with other 'steps' and build up to a propagated impulse (see Fig. 7). Once a full-size spike, whether afferent or antidromic, has arisen, it apparently invades all the terminal branches and so produces a period of complete silence (p. 256). A miniature spike which fails to propagate cannot render the other terminal units refractory, and this probably explains the absence of a silent period in these instances.

DISCUSSION

The 'negative after-potential' at the nerve endings

It has been pointed out that this effect might be caused by a gradient of membrane polarization along the terminal branches, the endings being at a higher resting potential than the trunk of the axon. It is well known (Schaefer, 1934) that the action potential becomes longer and is followed by a 'negative after-potential' when it travels through an anelectrotonic, i.e. more strongly polarized, region. The characteristic difference between afferent and antidromic spike could, therefore, be explained if the peripheral end of the fibre were more strongly polarized and thus normally in an 'anelectrotonic' state. Unfortunately, the present preparation does not lend itself to a direct test of this matter, for with a spike amplitude of 2–3 mV., a resting potential difference of less than 1 mV. would have to be looked for, and a steady potential difference of this size is impossible to measure in the presence of cut nerve branches.

It might be argued that a steady potential difference and, therefore, the triphasic action potential may arise, not because of some special property of the nerve endings, but perhaps because the region of the fibre near the *central* recording electrode might have been depolarized by injury currents from the cut peroneal nerve (cf. Fig. 2). This suggestion, however, can be dismissed because the presence of the third phase depended upon the proximity of a spindle to the peripheral lead. The local after-potential was not seen in fibres which did not terminate near-by, although they must have been equally affected by any injury currents from the cut peroneal branches.

The propagation of 'new-born' impulses from the spindle

It might be suggested that an anelectrotonic gradient along terminal branches would also account for the apparent obstacle which bars the propagation of newborn afferent impulses and for the 'notches' and 'steps' in Figs. 3–5 and 8, which resemble those described by Erlanger & Blair (1934) during partial anodal block. But there is another and perhaps simpler way of explaining these effects. Before the sensory axon terminates it divides into several small branches (cf. Fig. 1B). It is conceivable that the transmission of an impulse from a fine terminal, or pre-terminal, branch into the main axon occurs with a lower safety margin than in the reverse direction. A sudden increase of diameter, at some points of bifurcation, would cause the afferent action currents to be reduced in density, and the geometrical disparity between a fine and coarse adjoining portion of the axon cylinder may account for different facility of propagation in the two directions, and, in extreme cases, even for a one-way block. This is speculative, but of some general interest because similar events would be likely to occur at the dendrites of central neurones. Whatever the correct explanation, the quantal size and the brief time course of the observed 'step-potentials' distinguish them sharply from any graded local potential such as that described in the following paper. There is little doubt that the two or three

local 'steps' shown above are true spikes which have started independently at terminal points of the spindle, and have yet to overcome some physiological obstacle before a sensory message is originated.

The 'random discharge' at low tension

An interesting question is posed by the large and apparently random variations in the frequency of the afferent discharges when the tension of the muscle is very low. There is no evidence of a regular rhythm in either propagated or local action potentials (Figs. 6, 8). Apparently, there are considerable fluctuations in the excitatory state of the stretch receptor, but their cause is at present unknown. It is pertinent that the local abortive spikes form part of this 'random discharge' at low tension, and are replaced by a regular pattern of propagated impulses when the tension is raised. It has been argued that abortive impulses arise from fractional activity within the spindle, that is from a separate excitation of individual groups of receptor terminals. If that argument is accepted, it would follow that the irregular disturbances which become so evident at low tension are due to highly localized events which take place at individual endings within the spindle. It is not inconceivable that molecular agitation in the mechanical receptor substance, or ionic noise in the terminal nerve membrane is ultimately responsible for the observed fluctuations in the local excitatory level.

SUMMARY

1. Sensory impulses from frog muscle were recorded at a point close to the spindle. The shape of these impulses differs in two respects from the ordinary axon spike: (*a*) there is a localized 'negative after-potential' at the nerve endings; (*b*) afferent spikes are initiated by brief step-like 'pre-potentials' which at times remain abortive.

2. The negative after-potential at the nerve endings follows the afferent as well as the antidromic spike and persists for several milliseconds, at 20° C.

3. At low levels of tension, successive spikes in the same axon show characteristic differences in shape. Two or three discrete patterns of 'pre-potentials' can be distinguished which recur consistently throughout the experiment. In many preparations, 'pre-potentials' are observed which occasionally fail to propagate and then form small monophasic spikes of discrete 'quantal' sizes. Such abortive spikes are wiped out by antidromic bombardment of the spindle at a rate of about 100 per sec., and by moderate stretch which gives rise to a regular series of full-size afferent impulses.

4. The presence of step-like pre-potentials and of abortive spikes indicates that there are local obstacles which delay and sometimes block the propagation of 'new-born' impulses after they have arisen at the receptor terminals. It is suggested that transmission from a fine terminal branch into the axon trunk

has a lower safety margin than transmission in the reverse direction, and that a delay or partial block might occur at some points of bifurcation of the sensory nerve fibre.

I wish to thank Prof. A. V. Hill for the facilities provided in his laboratory and Mr J. L. Parkinson for his invaluable assistance. I am indebted to Prof. J. Z. Young and the staff of his department, in particular to Mr F. J. Pittock and Mr J. Armstrong, for frequent help.

REFERENCES

Arvanitaki, A. (1939). *Arch. int. Physiol.* **49**, 209.
Erlanger, J. & Blair, E. A. (1934). *Amer. J. Physiol.* **110**, 287.
Katz, B. (1949). *J. exp. Biol.* **26**, 201.
Katz, B. (1950). *J. Physiol.* **111**, 261.
Lorente de Nó, R. (1947). *A Study of Nerve Physiology*, **1** and **2**. In *Stud. Rockefeller Inst. med. Res.* **131** and **132**. New York.
Matthews, B. H. C. (1931). *J. Physiol.* **72**, 153.
Schaefer, H. (1934). *Ergebn. Physiol.* **36**, 151.
Young, J. Z. (1950). In preparation.

J. Physiol. (1950) 111, 261–282 612.88.074.612.743

DEPOLARIZATION OF SENSORY TERMINALS AND THE INITIATION OF IMPULSES IN THE MUSCLE SPINDLE

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It has been shown by several authors (Adrian & Gelfan, 1933; Fessard, 1936; Arvanitaki, 1938; Hodgkin, 1948) that the rhythmic response of a muscle or nerve fibre to an electrical or chemical stimulus is initiated by a local preliminary depolarization which re-develops after each discharge and which varies with the strength of the stimulus. It has been suggested that similar local potential changes intervene between the various forms of sensory stimuli and the initiation of afferent impulses (Pantin (1937, p. 418); Granit's 'generator potential' (1947); Stevens & Davis's 'transducer effect' (1938)). The existence of local potential changes in sense organs, e.g. the electro-retinogram, or the cochlear microphonic effect, has been known for many years, but their exact significance and relation to the discharge of impulses has not been established with certainty. Recently, important evidence was obtained by Hartline & Graham (1932), by Granit (1947) and by Bernhard (1942), who showed that visual stimulation of the eye gives rise to electrotonic potentials in the optic nerve (see also Parry, 1947). In the present paper, further evidence is presented using the muscle spindle of the frog. It will be shown that stretching of the muscle produces a depolarization of the sensory nerve endings which spreads electrotonically along the axon and varies directly with the rate and amplitude of the mechanical stimulus. By a suitable dose of a local anaesthetic the sensory nerve impulses can be abolished and the spindle potential obtained separately. The local spindle potential appears to be an essential link between input and output of the sense organ, and the mechanism whereby a mechanical deformation is converted into this electrical membrane change will be discussed.

METHOD

Preparation and method of recording have been described in preceding papers (Katz, 1949*a*; 1950*b*). The electrode arrangement is shown in Fig. 1. To apply a synchronized stretch to the muscle, two procedures were used. In some experiments where only qualitative information was required the preparation was stretched by stimulating another frog muscle which had been placed in series with the preparation. Usually, however, one tendon was connected via a lever to a magnetic relay. The

velocity of stretching was adjusted by varying the energizing current or a viscous resistance which opposed the motion of the lever. The mechanical excursion could be registered together with the electric response of the sensory axon on a double-beam oscilloscope. The movement of the lever was recorded without noticeable delay by using a photocell with cathode follower output.

Several checks were made to discriminate against electrical artifacts which might accompany stretching of the muscle. Such artifacts could arise from resistance changes in the presence of grid current, but tests with a blocking condenser at the input (Katz, 1950*b*) showed that this effect was quite negligible.

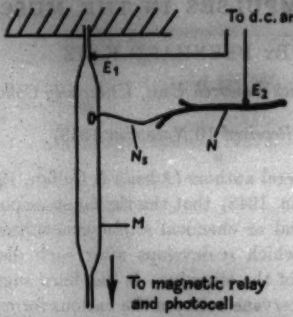

Fig. 1. Electrode arrangement. *M*, M. extensor longus dig. IV.; N_s, isolated sensory axon; *N*, peroneal nerve.

A more serious possibility of error arose from the fact that a portion of the muscle was included between the recording electrodes (Fig. 1), and any potential change occurring in the muscle would vitiate the experiment. Artifacts of this kind were checked by short-circuiting the sensory axon (letting it lie along the muscle) or by crushing it at the point of entry into the muscle (see, for example, Figs. 7 (3); 9 (7)). In this way, electric potential differences in the sensory nerve were eliminated, and artifacts due to the muscle were recorded alone. In many preparations, the potential changes were abolished by this procedure, and this showed that the electric response to stretching originated wholly in the spindle. In some instances, however, small residual potential changes in the muscle were observed, and it was then safest to discard the experiment altogether. Artifacts of this kind appeared to be due to local injury or to tissue debris which had been left on the muscle. The best way of avoiding this difficulty was clean and careful dissection.

It is worth mentioning that in a few experiments an intact motor axon was isolated instead of a sensory fibre. In these cases, no potential change could be recorded while the muscle was stretched, and this provided a satisfactory additional check.

RESULTS

Qualitative observations

Fig. 2 shows the electric response in the peripheral end of a sensory axon during a transient lengthening of the muscle. The stretching was produced by the contraction of a sartorius muscle to which single shocks of varying strength were applied. Records 2–4 were obtained with increasing shock intensity, 'diphasic' as well as 'monophasic' records being shown. During 'rest' there were occasional discharges of either propagated impulses showing the usual

Fig. 2. Potential changes in a sensory axon when the muscle is subjected to a transient stretch. The stretch was applied by a sartorius muscle contracting against the M. extensor dig. IV. All records in this paper read from left to right and show 'spindle negativity' (i.e. a positive potential difference between electrodes E_2 and E_1) as a downward deflexion. A: usual recording from uninjured axon. 1, at 'rest'; 2–4, with increasing intensity of stretching. B: the records have been made monophasic by crushing the central portion of the axon. *Note.* In this preparation, the axon divided into two branches before entering the muscle. Occasional alternations in spike size (e.g. record A, 2) are probably to be attributed to impulses starting along alternate branches of the axon.

Fig. 3. Occasional 'block' during intense depolarization. Same experiment as in Fig. 2. The places where an impulse has dropped out are marked by arrows.

triphasic pattern or of small abortive impulses (Katz, 1950*b*). During the stretching, a burst of propagated spikes occurred, superimposed on a slow depolarization which was localized at the peripheral lead. The amplitude of this depolarization and the frequency of the spike discharge increased with the intensity of the mechanical stimulus.

It is interesting to note that during the intense depolarization in Fig. 2, record 4, the size of the spike diminished. This cannot be attributed simply to the refractory state left behind by preceding impulses, for the *diphasic* record indicates that, after conduction to the proximal lead a few millimetres away, the spikes have reached their normal amplitude. The reduction in spike height must be the result of the local depolarization—an effect which has previously been described as a cathodic depression (e.g. Hodgkin, 1948; Lorente de Nó, 1947). This depressing action can become so intense that spikes fail to propagate (see Fig. 3), so that during a vigorous stretch impulses may appear to drop out of an otherwise regular series.

A curious fact, invariably observed, was the marked difference in size between the small initial depolarization which precedes the first impulse and the conspicuous potential change leading up to the second and later impulses of a series. In fact, in some experiments (e.g. Fig. 4B), no distinct step could be seen preliminary to the first discharge, while there was always a large depolarization preceding the later impulses. The significance of this observation will be discussed at a later stage (pp. 273–4), but it is necessary to dispose at once of an important objection. It might be argued that the local depolarization in Fig. 2 is not the direct result of stretching, but a cumulative effect of after-potentials left by a train of nerve impulses. It has previously been shown that the spike is followed by a local negative after-potential at the nerve endings (Katz, 1950*b*), but it can now be shown that the depolarization during stretching is a separate phenomenon: (*a*) in many experiments (e.g. Figs. 2, 3 and 4A), there is no doubt that a small initial depolarization occurs *before* the first spike arises; (*b*) the depolarization builds up gradually during the interval between spikes *after* the negative after-potential has declined (Figs. 2, 4–6 and 12); (*c*) spikes and after-potential can be eliminated by a local anaesthetic without affecting the depolarizing effect of stretching (p. 269); (*d*) when an intense depolarization has been produced by stretching (Figs. 4 and 5), the negative after-potential becomes reduced or even disappears. Evidently we are dealing with a direct action of the mechanical stimulus on the nerve membrane, without the intervention of a nerve impulse.

Dynamic and static stretch effects

The time relations between stimulus and response were examined by using a double-beam oscilloscope and recording the change of length of the muscle simultaneously with the electric changes in the sensory nerve.

In Fig. 4B, an experiment is illustrated in which a small test stretch, of 1·3 mm. amplitude, was applied at various speeds to a muscle of 17·5 mm. initial length. There is a discharge of nerve impulses superimposed on a slow

depolarization. The most striking phenomenon, however, is the apparent separation of the electrical changes into two phases: (*a*) a relatively intense dynamic effect which coincides with the period of initial lengthening, and (*b*) a final static effect during which the local depolarization and the rate of discharge are maintained at a lower level (see also Figs. 6, 7 and 9, and Katz, 1949*b*).

Fig. 4. Double-beam recording of mechanical lengthening (upper trace) and electric potentia changes (lower trace). A, fast time base. Monophasic recording. Initial muscle length 16 mm. B, another preparation. Slower time base, Diphasic recording. Initial length 17·5 mm. Occasionally an impulse from the 'resting spindle' is superimposed on the record of the stretch response. Each record consists of two exposures, (i) a 'base-line' (with an occasional impulse appearing on it) and (ii) the stretch response.

It is noteworthy that the discharge of impulses begins as soon as stretching commences; in fact, the first impulse is often discharged when the mechanical record has barely risen from its base-line. It should be remembered, however, that the 'resting' spindle is on the verge of firing and that, therefore, the initiation of the first impulse may require only a minute stimulus.

The intensity of local depolarization can be measured during the interval between impulses. This measurement is somewhat arbitrary because the potential does not remain at a steady level. In practice, the 'flattest' portion

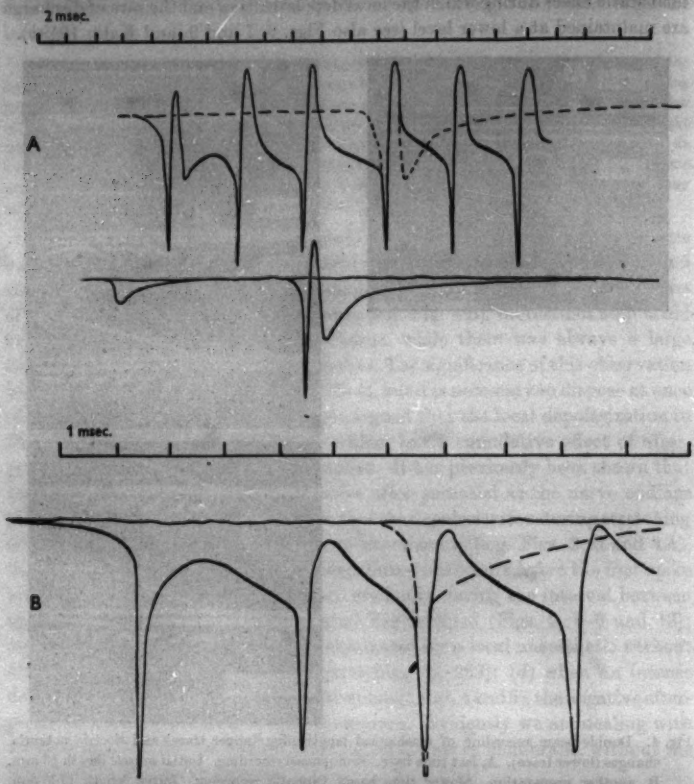

Fig. 5. Stretch response and resting discharge in a sensory axon. Note the reduction of the negative after-potential during stretching. A, upper record: continuous tracing shows the response to stretching. Broken line tracing shows an impulse from the 'resting' spindle. Lower record shows an abortive and a propagated spike from the 'resting' spindle. B, stretch response (continuous tracing) and 'resting' discharge (broken line) in another preparation. Records were monophasic in this instance.

of the curve between successive spikes was chosen for measurement and mean values were determined in each record (*a*) for the 'dynamic' and (*b*) for the 'static' depolarization (see also Katz, 1950*a*). The 'dynamic' effect with various

rates of stretching is plotted in Fig. 8. These results were taken from a single preparation; in seven other experiments similar results were obtained. The

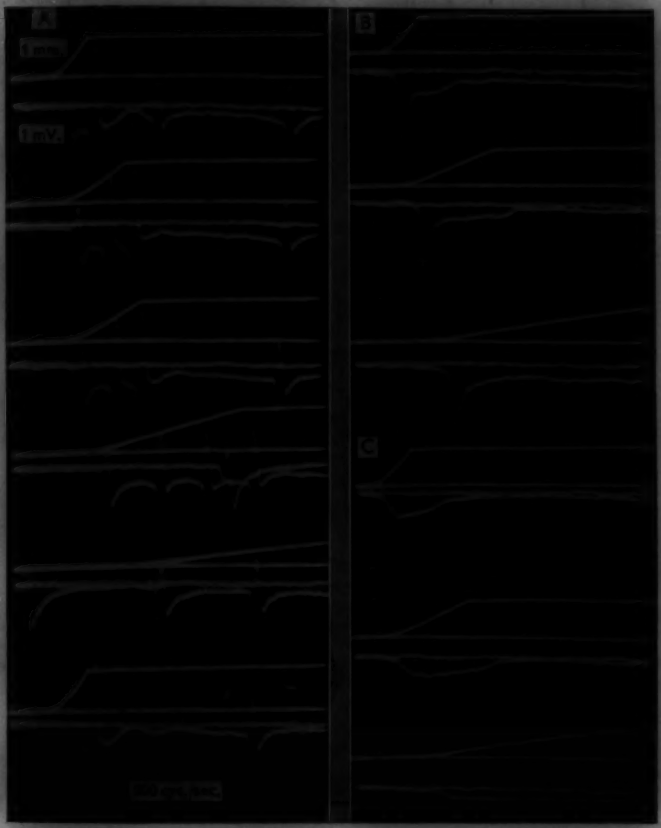

Fig. 6. Effect of procaine on the stretch response. A, normal preparation. Various rates of stretching. B, after application of 0·25% procaine. C, after application of 0·5% procaine. Note the initial non-propagated spike in B.

depolarization increases with the velocity of stretching up to a maximum which, in the case of Fig. 8, was 0·75 mV., or about 20% of the recorded spike height. When the muscle was stretched at a rate greater than 1–1·5% per msec.

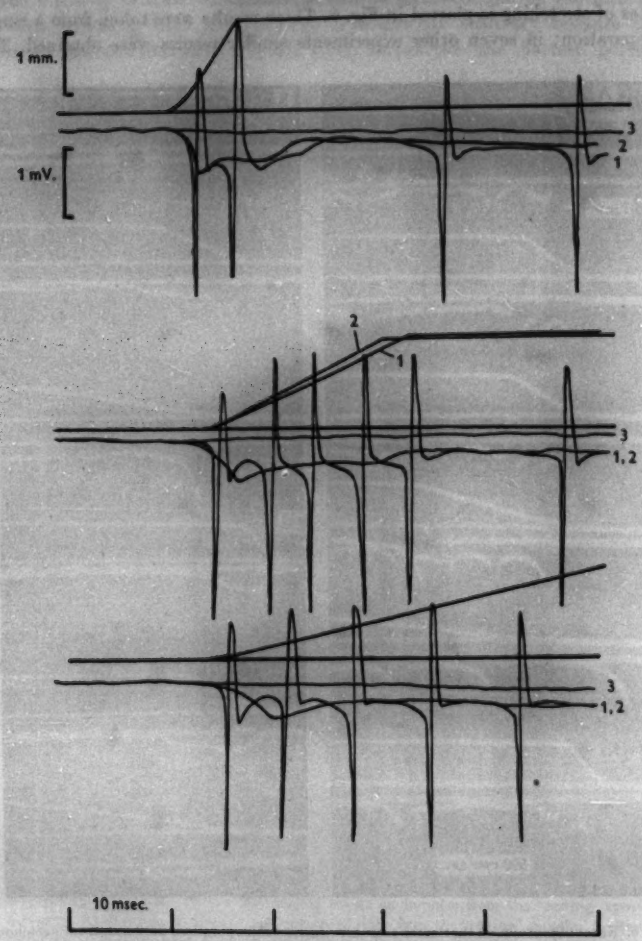

Fig. 7. Superimposed tracings of stretch responses before and during local anaesthesia. Three different rates of stretch shown approximately in the upper drawings. The electrical potential changes were recorded (1) in the normal preparation, showing repetitive spikes and local depolarization; (2) after application of 0·35 % procaine showing only local depolarization (with a trace of an initial local spike); (3) after crushing the axon at its point of entry into the muscle, the record becoming now indistinguishable from a base-line.

no further increase in the local depolarization occurred. A half-maximal effect was produced by stretching at a rate of 0·28% per msec. (mean of eight experiments, at 16–20° C.: 0·23% per msec., S.E. of mean ±0·034% per msec.). The 'static' effect of a 1·3 mm. extension (about 8% of muscle length) amounted to 0·26 mV.: an equivalent 'dynamic effect' was produced by stretching the muscle at a rate of 23 μ. (0·15% of its length) per msec.

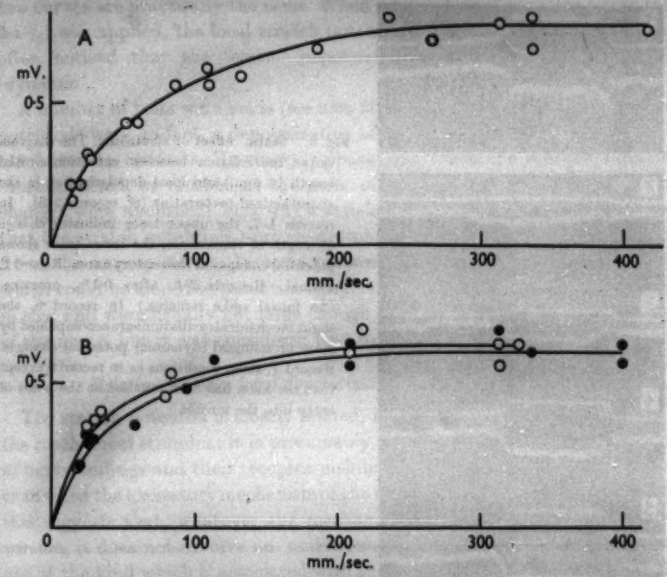

Fig. 8. 'Dynamic' effect of stretching. Initial length of muscle 15·5 mm. Abscissae: rate of stretching of muscle. Ordinates: local depolarization. A, normal preparation. B, anaesthetized preparation. Hollow circles, 0·25% procaine. Full circles, 0·5% procaine.

The effect of local anaesthetics

When the preparation was soaked for several minutes in a buffered solution of procaine/Ringer, the impulse discharge was curtailed or abolished, according to the concentration of the anaesthetic. With 0·1% procaine, a single propagated impulse usually remained: with 0·2% there was evidence of a residual non-propagated spike: with higher concentrations all signs of excitatory activity usually became extinguished while the prolonged depolarization during stretch was either unaffected (with concentrations up to 0·3%) or somewhat reduced (0·4–0·5%). Similar effects were seen when the preparation was treated with a sodium-free solution (0·12 M/l. choline chloride plus the usual

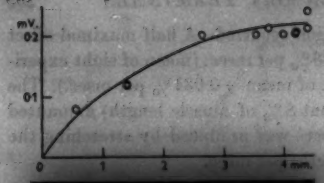

Fig. 9. 'Static' effect of stretching. The diagram shows the relation between extension (initial length 16 mm.) and local depolarization in the anaesthetized preparation (cf. records 2–6). In records 1–7, the upper trace indicates change of length of the muscle, the lower trace shows potential changes in the sensory axon. Record 1, normal. Records 2–6, after 0·1% procaine. (An initial spike remains.) In record 6, the slight mechanical oscillations are accompanied by more pronounced (dynamic) potential changes. Record 7, same conditions as in record 6 except that the axon has been crushed at the point of entry into the muscle.

amounts of KCl and $CaCl_2$). After soaking for 10 min. in this medium, a single impulse was seen instead of a burst of discharges; after a further 30 min. immersion, all traces of spikes, local and propagated had vanished while the prolonged depolarization was still obtained.

In Figs. 6 and 7 the effect of procaine is illustrated, and in Fig. 8A and B, the 'dynamic stretch potentials' are plotted before and after paralysis. The two curves are practically the same. When a large dose of procaine (more than 0·4%) was applied, the local stretch potentials diminished in size, and it was often noticed that the 'static' depolarization diminished more than the 'dynamic'.

A number of tests were made (see also Method) to ascertain that the stretch potentials were, in fact, a depolarization of the sensory axon and could not be attributed to artifacts. In many preparations, when the sensory axon was short-circuited, or crushed at the point of entry into the muscle, all electric changes were abolished. This was a satisfactory test and confirmed the view that the stretch potentials were electrotonically conducted into, and recorded from, the sensory nerve. In some preparations electrical artifacts were seen which could be traced to potential differences in the muscle tissue; they varied in sign and were usually not large enough to mask the genuine depolarization of the nerve, but it was considered best to discard such experiments.

Relation between local depolarization and impulse discharge

The stretch potential is closely related, in its time course and amplitude, to the mechanical stimulus: it is presumably the direct result of the deformation of nerve endings and their receptor membranes. The potential change persists even when the excitatory mechanism of the nerve membrane has been paralysed: this suggests that, whatever the mechanism of the electro-mechanical conversion, it does not involve an 'active response' of the nerve endings, at least not of the kind which is associated with the action potential. The discharge of nerve impulses appears to be a secondary phenomenon, initiated by the stretch potential in a manner analogous to electric excitation in nerve or muscle.

This view is supported by the close relation which exists between the intensity of the local depolarization and the frequency of impulses. The relation is obvious from the records, and was tested statistically in one experiment, illustrated in Fig. 10. The intervals between successive impulses were measured as well as the level of depolarization during the intervals. There was a highly significant correlation with a coefficient of 0·97 (ninety-one pairs of observations). The regression line in Fig. 10 was fitted by the usual statistical method and shown to have a highly significant slope.

There is an interesting analogy between the discharge from a muscle spindle and the repetitive response to constant current in a crustacean nerve fibre (Fessard, 1936; Hodgkin, 1948; see also Fig. 11). In both instances, a con-

tinuous stimulus gives rise to rhythmic impulses whose frequency depends upon the stimulus strength. Furthermore, both types of response are mediated by a local depolarization. There are, however, noticeable differences. In the crustacean axon, the level of the depolarization from which successive spikes

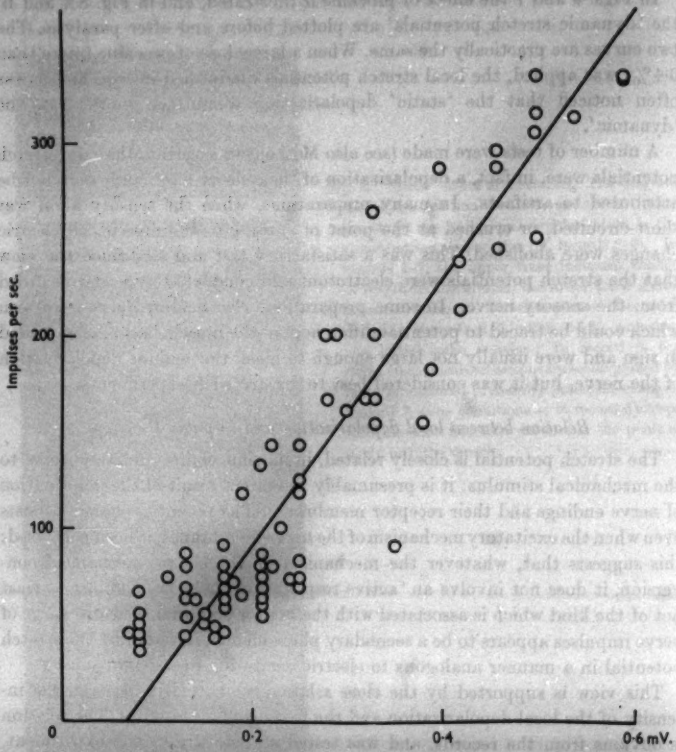

Fig. 10. Relation between local depolarization (abscissae) and frequency of impulses (ordinates). A regression line has been drawn through the results which were obtained from ninety-one pairs of observations. For further explanation see text.

take off remains almost constant: it is practically the same for the first as for the following spikes of a series of impulses, and does not vary much with the frequency of the discharge. In the spindle axon, however, the local depolarization which precedes the first of a series of impulses is always small, and the 'firing level' increases markedly with the frequency of the impulses. An ex-

planation of this difference may be found along the following lines. The recovery from a nerve impulse involves two changes: (i) a restoration of the membrane resistance, and (ii) a gradual return of 'excitability'. The existence of two different processes is indicated by the fact (Hodgkin, 1938) that (1) a stronger current is required during the refractory period to build up a given potential change across the membrane, and (2) a larger potential change is required to evoke a membrane response. How these two factors are coupled is not yet

Fig. 11. Response of *Carcinus* nerve fibre to electric currents, recorded at the cathode (cf. Hodgkin, 1948). The numbers indicate relative current strength.

known; they may depend upon different mechanisms, and there is no reason to suppose that they proceed at identical rates or even at the same relative rates in different tissues. If the repair of the membrane leakage were considerably quicker than the return of excitability, a behaviour as seen in the spindle axon would be obtained, for a larger local depolarization would be required before the second, third, etc. impulse of a series could be initiated. If, on the other hand, both recovery processes occurred at approximately the same rate, or the second one were faster, then all the impulses would take off at practically the same level as is found in the *Carcinus* axon.

In this way it seems possible to explain the differences between the electrotonic stretch effects at the spindle and the cathodic potentials in isolated crustacean axons. The depolarization, which precedes the first of a series of sensory impulses, is small, probably because the threshold of the normal 'rested' nerve terminals is low. It should be remembered that the normal 'resting' spindle is on the verge of firing (it does, in fact, discharge impulses at a low irregular rate), and that therefore the initial impulse during stretch may be set up by a very weak stimulus and a very small local depolarization, just enough to trigger the most excitable nerve terminal. The next impulses are initiated at a time when all the nerve endings are relatively refractory, and a much larger depolarization is then required to give rise to a spike. Similarly, a large local stretch potential is obtained when the threshold has been raised by other means, e.g. by the application of an anaesthetic. Thus, the observed relation between local and propagated changes can be explained in simple terms, and there is no reason to suppose that the sensory impulses arise from any agent other than the local stretch potential.

Sensory potential changes accompanying the release of a stretched muscle

It has been shown by Matthews (1931*b*) that during the release of a stretched muscle the discharge of impulses from a spindle drops below its final steady rate immediately after the tension is reduced. It was of interest to investigate the

Fig. 12. 'Off-effect' at the end of a period of stretching. Note the appearance of a miniature spike in A 2 and B 1.

local potential changes which accompany this phenomenon. When a normal muscle is released, the depolarization in the sensory axon suddenly falls and the discharge of impulses stops (Fig. 12). This 'cut-off' occurs without noticeable lag; the depolarization falls, in fact, so quickly that a local 'miniature spike' is formed when the mechanical release happens to coincide with the preliminary rise which precedes the firing of an action potential (Fig. 12). There is evidence for a transient *positive* potential change, i.e. a dynamic 'off-effect'. Examples are shown in Fig. 13. The positive spindle potential is usually smaller

and more prolonged than the 'on-effect', but this is to be expected, for the mechanical conditions during release are not the exact converse of those during forcible extension. The speed at which the sense organ returns to its initial shape depends upon its own elastic restoring forces and viscous resistances rather than upon the speed of the applied lever and, therefore, the effects of stretch and release can be compared only qualitatively.

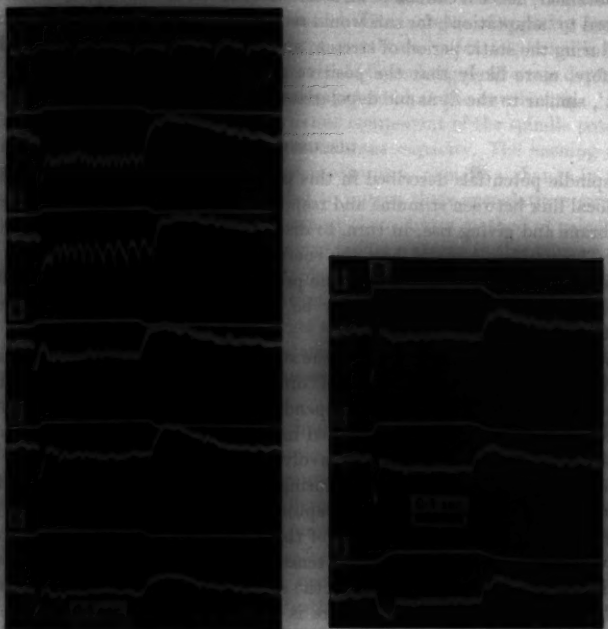

Fig. 13. 'Off-effect' at the end of a period of stretching. A, initial length 15–16 mm. In records 2–6 a 1·8 mm. stretch was applied. Records 1–3, normal preparation, initial length being slightly less in 3 than in 1 and 2. Record 1 shows 'resting' discharge. Records 4 and 5, after application of 0·15% procaine; record 6, after 0·3% procaine. (In records 4 and 5 an initial spike was present, but is not visible in the reproduction.) B, another preparation, treated with 0·3% procaine. Initial length 15 mm. Three stretches of 1·3 mm. amplitude, applied at different rates. (In B 1 an initial spike was set up in the region between the recording leads.)

The 'off-effect', in Fig. 13, might be interpreted in various ways: (*a*) it might be an after-potential left behind by the preceding train of impulses; (*b*) there is 'adaptation' during the static period of stretch shown by the gradual fall in the discharge rate: if 'adaptation' were due to some process opposing the

local depolarization, a positive 'off-effect' could appear as a consequence; (c) it might be the counterpart of the dynamic 'on-effect' as suggested above, associated with a relatively slow return of the sensory structure to its initial length.

It is possible to decide between these suggestions by the use of procaine (Fig. 13). In the absence of sensory impulses, the positive potential change is still obtained; hence it cannot be an after-effect of nerve activity. Nor can it be ascribed to 'adaptation', for this would require a slow decline of the depolarization during the static period of stretch, and no such decline is observed. It is, therefore, more likely that the positive potential change is a dynamic 'off-effect', similar to the dynamic depolarization described above (p. 265).

DISCUSSION

The spindle potentials described in this paper can be satisfactorily explained as a local link between stimulus and response, arising in the terminal receptor membrane and giving rise, in turn, to discrete messages in the attached nerve fibre. The problem remains how the energy of the mechanical stretch is converted into a local depolarization. The present experiments provide no direct evidence on this matter, but it may be of interest to consider some simple hypotheses.

If we compare the time courses of the stimulus and the resulting depolarization, it is clear that a certain degree of 'differentiation' has taken place, a large component of the spindle potential depending upon rate rather than magnitude of stretch. This might be accomplished in different ways and two factors will be discussed both of which may be involved.

The 'differentiation' could occur during the mechanical transmission of the stimulus from the tendon to the receptor membrane (see Matthews, 1931*a*). For example, if the sensory portion of the spindle were less viscous than the muscular portion in series with it, the sense organ would suffer greater deformation during the dynamic than during the static phase of stretching. While this possibility cannot be dismissed, there is another way of explaining the differentiation of the time course as well as the origin of the spindle potential.

When the polarized surface membrane of a nerve or muscle fibre is stretched, electrical changes may arise in several ways: first, there may be a transient fall in the membrane potential while the membrane is being stretched and its capacity increased (cf. Ramsey, 1947). This action would be similar to that of a 'condenser microphone'. If we knew the time constant of the membrane, its resting potential and rate of stretching, the resulting depolarization could be calculated as shown in the Appendix. Secondly, the membrane permeability may be altered by stretching, ionic pores, for instance, might be enlarged and consequently the resting potential may fall. Thirdly, chemical changes may take place in the interior of the cell as a result of stretching (cf. Feng, 1932), and

these may subsequently affect the distribution of ions and the potential across the surface membrane. The capacitative effect seems almost inevitable, provided that the membrane is initially in an expanded state. If it were not, stretching would merely straighten out some folds without altering the electrical properties. But the surface area of an expanded membrane would increase and its thickness diminish when it is stretched, and one would therefore expect that the capacity (C) of the membrane becomes greater, and its transverse resistance (R) less, as the fibre is lengthened. The increase of capacity must be accompanied by an immediate fall of the membrane potential; but this would be a transient effect and the membrane would be recharged, at a rate depending upon its time constant RC, to its resting potential at the new length.

Thus, it is conceivable that the dynamic component of the spindle potential is simply the result of a change in membrane capacity. The ensuing static component may be due to a different mechanism, namely a change in membrane permeability. It is not intended to pursue these speculations seriously until more evidence is available to support them, but a method of calculating the 'dynamic component' of this receptor model is shown in the Appendix. As a point of interest, the observed depolarization is too great to be explained without some mechanical amplification of the applied stretch. Unless the deformation of the receptors were larger than that of other parts of the muscle, the present hypothesis would be untenable, but this difficulty would be removed, if the sensory structure of the spindle were more 'compliant' than the rest of the intrafusal bundle.

It may be objected that this hypothesis unnecessarily invokes two different mechanisms for the dynamic and static response. This assumption, however, is not entirely unfounded. There were indications in several experiments that the two components are affected differently by large doses of procaine, the static potential change being reduced more drastically than the dynamic change. Furthermore, the discharge of impulses frequently occurs in two discrete groups, corresponding to the dynamic and static phases of stretch (cf. Matthews, 1931*a*). This is seen in Fig. 7 and further illustrated in Fig. 14. There is a gap between the two groups of impulses, and this gap corresponds to a 'dip' in the stretch potential observed in the procaine-treated preparation (Fig. 7). This would agree with the suggestion of two separate components of the potential change, the 'static' effect developing more slowly and at times rising after the quick 'dynamic' effect has already begun to decline. These observations are not conclusive but they would fall into line with the idea of two separate components of the stretch potential, and would be more difficult to explain on the idea of a simple mechanical differentiation (p. 276).

The mechanism of the stretch receptor suggested here does not invoke any specific properties other than those found in nerve or muscle fibres. The factors which distinguish mechanical receptor endings from ordinary nerve or muscle

membranes may be of a rather simple nature: first, as suggested above, the nerve endings may be subject to greater deformation than the adjacent tissue. Secondly, the surface membranes of muscle fibres, and of nerve axons *in situ*, may not be in the fully extended state without which the theoretical mechanism could not work (cf. Katz 1950*a*). Finally, the threshold at the nerve endings might be lower than elsewhere so that a relatively small amount of depolarization would be sufficient to start an impulse. While such factors would suffice

Fig. 14. The 'gap' between the dynamic and static discharge of impulses. A and B, two different preparations. Amplitude of stretching: A, 17%; B, 8% of the initial length.

to explain the differentiation of the spindle receptors, one cannot dismiss the possibility of a specially adapted molecular mechanism, for example some piezo-electric substance, being responsible for the conversion of stretch into a spindle potential. The problem thus remains whether muscle spindles and mechanical receptors generally work on a specific molecular basis, like most photo- and chemo-receptors, depending upon specific chemical reactions, or, as suggested here, like an ordinary electro-mechanical converter system, as condenser microphones or resistance strain gauges.

SUMMARY

1. When a frog muscle is stretched, its sensory nerve endings become depolarized, and a local potential change can be recorded from the sensory axon at a point close to the spindle.

2. This potential change varies with the rate and amplitude of stretching, and gives rise to repetitive impulses in the sensory nerve.

3. By applying a local anaesthetic it is possible to abolish the nerve impulses without affecting the local electric reactions to stretch. There is evidence for two distinct components of the potential change associated with the dynamic process of stretching and with static extension respectively.

4. When a stretched muscle is released, a transient potential change in the opposite direction (i.e. a positive variation at the nerve endings) is observed.

5. The local potential change appears to arise from a direct action of the mechanical stimulus on the sensory nerve endings and to be a link between the mechanical input and the rhythmic output of impulses from the sense organ. The mechanism whereby the mechanical deformation is converted into an electrical membrane change is discussed.

APPENDIX

The immediate effect of stretching on an electrically charged membrane may be represented by a simple model (Figs. 15, 16). A membrane element of capacity C_0 and conductance G_0 ($=1/R_0$) is normally charged to the resting potential E.

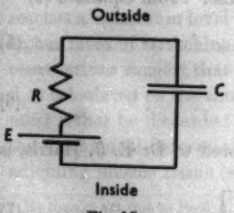

Fig. 15.

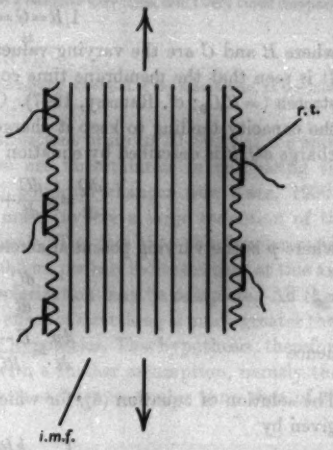

Fig. 16.

Fig. 15. Electrical model of the receptor membrane.

Fig. 16. Schematic diagram of intrafusal muscle fibre (*i.m.f.*) with several receptor terminals (*r.t.*) on its surface. Arrows indicate direction of stretching.

When the tissue is stretched, the volume of the stretched elements remains constant but their surface area increases and thickness diminishes (provided that these elements are fully extended and their surface membrane is in an unfolded state). The terminal membrane of the sensory nerve is assumed to change in this manner when the muscle is stretched.

If it be assumed further that the dielectric constant and specific conductivity of the membrane material remain unaltered, then the total capacity and conductance of the membrane must become greater as its surface area increases and its thickness diminishes. In a cylindrical structure, area and reciprocal

thickness vary as the square root of length, hence the membrane capacity and conductance, being proportional to both variables, would change in direct proportion to the length.

If the muscle is subjected to a constant rate of stretching ('dynamic' phase), the length of the muscle varies according to

$$l = l_0 (1 + kt), \tag{1}$$

where l is varying length, l_0 the initial length of the muscle, t is time and k a velocity constant determining the rate of stretching. The changes in capacity and conductance of the membrane are then described by equations (2) and (3):

$$C = C_0 (1 + kt), \tag{2}$$

$$1/R = G = G_0 (1 + kt), \tag{3}$$

where R and C are the varying values of membrane resistance and capacity. It is seen that the membrane time constant RC remains constant during the stretch ($= R_0 C_0$; cf. Ramsey, 1947). Current will flow from the source E into the capacity tending to keep it charged to the resting potential. The flow of charge dQ/dt is described by equation (4):

$$\frac{dQ}{dt} = p\frac{dC}{dt} + C\frac{dp}{dt} = \frac{E-p}{R}, \tag{4}$$

where p is the varying potential across the membrane. From equation (2)

$$\frac{dC}{dt} = kC_0; \tag{5}$$

hence

$$\frac{dp}{dt} = \frac{E-p}{RC} - \frac{pk}{1+kt}. \tag{6}$$

The solution of equation (6), for which I am indebted to Dr E. J. Harris, is given by

$$p = E\left[1 - \frac{kRC}{1+kt}(1 - e^{-t/RC})\right] \tag{7}$$

for $p = E$, at $t = 0$. The 'dynamic depolarization' is given by $E-p$, and its relative value by $(E-p)/E$:

$$\frac{E-p}{E} = \frac{kRC}{1+kt}(1 - e^{-t/RC}). \tag{8}$$

To find this value we must know the time constant RC and the rate of stretching k. When k is made very small compared with $1/RC$, the depolarization, i.e. $(E-p)/E$, develops exponentially with time constant RC to a value given approximately by kRC, and with continued stretching it slowly declines. Tentatively, one may, therefore, derive an approximate value of RC from the initial development of the depolarization with low rates of stretch (p. 267, Fig. 6C), the result being of the order of 3 msec.

With a rate of stretching of 20 mm. per sec. and an initial length of 16 mm., k is 1·25 sec.$^{-1}$. The maximum depolarization in this case should be only 0·4% of the resting potential. Experimentally, a depolarization was observed which amounted to about 10% of the recorded spike, and the value at the terminal membrane must have been even greater on account of the inevitable attenuation in the terminal axon branches.

It is clear that with a total extension of 10% of the muscle length the membrane capacity, according to equation (2), could not increase more than 10% and, therefore, the depolarization could not exceed this value, no matter at what rate the muscle was being extended.

For very fast 'instantaneous' stretches at which k becomes very large, and t very small compared with RC, equation (8) reduces to

$$\frac{E-p}{E}=\frac{kt}{1+kt};$$

hence a 10% stretch (i.e. $kt=0{\cdot}1$) could reduce the resting potential of the membrane, at the most, by 9%.

In the experiments, however, depolarizations of up to 30–50% of the spike potential were found. Although there are uncertainties in comparing the amplitudes of local, and propagated, potential changes (see Katz, 1950*b*, Method), the observed stretch effect must involve a large reduction of the resting potential at the sensory terminals. The fact that the depolarization reaches a maximum level with rates of 0·2 m. per sec. indicates that at this and higher rates of stretching the local depolarization may be complete. All these observations suggest that the electrical effect of stretching is much greater than that calculated on the simple condenser hypothesis. This hypothesis, therefore, must either be discarded or coupled with a further assumption, namely that the mechanical deformation of the nerve endings is greater than that of the adjoining muscle tissue (see p. 277).

My thanks are due to Prof. A. V. Hill for the facilities provided in his laboratory and to Mr J. L. Parkinson for his invaluable assistance. I am also indebted to Dr E. J. Harris and Mr D. A. Scholl for frequent discussion and advice.

REFERENCES

Adrian, E. D. & Gelfan, S. (1933). *J. Physiol.* **78**, 271.
Arvanitaki, A. (1938). *Les Variations Graduées de la Polarisation des Systèmes Excitables.* Paris.
Bernhard, C. G. (1942). *J. Neurophysiol.* **5**, 32.
Feng, T. P. (1932). *J. Physiol.* **74**, 441.
Fessard, A. (1936). *Recherches sur l'Activité Rythmique des Nerfs Isolés.* Paris.
Granit, R. (1947). *Sensory Mechanisms of the Retina.* London.
Hartline, H. K. & Graham, C. H. (1932). *J. cell. comp. Physiol.* **1**, 277.
Hodgkin, A. L. (1938). *Proc. Roy. Soc.* B, **126**, 87.
Hodgkin, A. L. (1948). *J. Physiol.* **107**, 165.
Katz, B. (1949*a*). *J. exp. Biol.* **26**, 201.
Katz, B. (1949*b*). *J. Physiol.* **109**, 9 *P*.
Katz, B. (1950*a*). *Collogues Internat. C.N.R.S.* Paris.

Katz, B. (1950*b*). *J. Physiol.* **111**, 248.
Lorente de Nó, R. (1947). *A Study of Nerve Physiology*, **1** and **2**. In *Stud. Rockefeller Inst. med. Res.* **131** and **132**. New York.
Matthews, B. H. C. (1931*a*). *J. Physiol.* **71**, 64.
Matthews, B. H. C. (1931*b*). *J. Physiol.* **72**, 153.
Pantin, C. F. A. (1937). *Proc. Roy. Soc.* B, **123**, 397.
Parry, D. A. (1947). *J. exp. Biol.* **24**, 211.
Ramsey, R. W. (1947). *Ann. New York Acad. Sci.* **47**, 675.
Stevens, S. S. & Davis, H. (1938). *Hearing*. New York.

J. Physiol. (1950) 111, 283–303 612.014.424.081.595.3

THE EFFECT OF STIMULATION ON THE OPACITY OF A CRUSTACEAN NERVE TRUNK AND ITS RELATION TO FIBRE DIAMETER

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It has been shown (Hill & Keynes, 1949) that the opacity to white light of a crustacean nerve trunk undergoes a reversible change when the nerve is stimulated. The cause of this phenomenon was at first obscure, though the inclination was to ascribe it to some change in the optical properties of the axoplasm or membrane of the fibres. The possibility that the effect was due to a change in fibre size was at first not considered, but this would now seem to be a sufficient explanation of what is observed, and the evidence pointing in this direction is set out here. Further support for this view has been provided by showing that the single large fibre of the cuttlefish does actually undergo a change in diameter when it is stimulated (Hill, 1950).

METHOD

Apparatus. The apparatus for holding the nerve was designed to allow recording of the opacity change brought about by an alteration in the composition of the electrolyte solution surrounding the nerve (Figs. 1, 2). The nerve could also be stimulated and the action potentials recorded. In this way the opacity change caused by stimulation could be compared with the change due to a known swelling or shrinking of the fibres, brought about by altering the osmotic pressure of the surrounding medium.

The photocell arrangement is a differential one, the balancing being done by controlling the light entering one cell. The recording circuit is shown in Fig. 3. The nerve chamber was constructed by milling and drilling out a block of Perspex, and polishing the optical surfaces. The solutions to be passed through the nerve chamber were contained in glass reservoirs, held at such a height as to give a head of pressure of about 2 ft. The substitution of one fluid for another was made by turning a tap, the second fluid then replacing the first in a matter of minutes. The flow of experimental solution round the nerve was controlled at about 0·3 c.c./min. by a capillary resistance in the outflow tube, and was sufficiently rapid to allow the medium to be completely changed in a few minutes, but slow enough to avoid disturbing the nerve by turbulence: in this way the opacity could be recorded while the change of medium was in progress. The solutions were previously filtered through a Berkfeld filter, to remove minute particles which might lead to fluctuations in the transmitted light. The solutions were oxygenated. Recording was done by hand from the galvanometer, using a stop-watch for timing. The nerve was stimulated for 10 or 15 sec. at 50 or 100 impulses per sec. with a multi-vibrator unit: the action potentials were observed on a Cossor oscilloscope (model 339A).

It was found necessary to pay particular attention to two features in the design of the apparatus: in the first place, the light beams must not traverse long air paths, for variations in the refraction of the air due to the heat of the lamp may lead to considerable fluctuations in the reading. In the present apparatus the rapid mixing of the air in the lamp housing, due to the constant stream of cooling air, prevents any such fluctuations which might arise from the movement of hot air close to the lamp; secondly, the different components of the system must have short, very rigid connexions

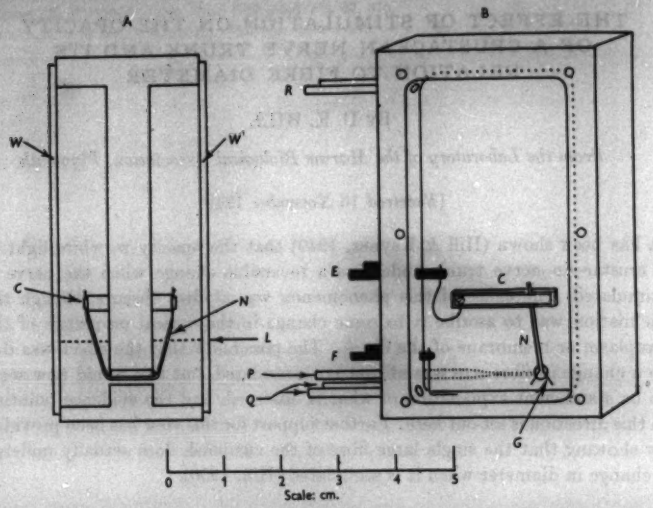

Fig. 1. The nerve chamber. A, vertical section through 'nerve tube'. *N*, nerve; *L*, level of sea water; *W*, window screwed on to side of nerve chamber after nerve is fixed in position (a rubber washer gives a watertight seal); *C*, clamp for holding nerve (also acts as electrode). B, side-view of nerve chamber. *N*, nerve; *C*, clamp for holding nerve (this is made of brass and allows electrical connexion with terminal *E*, for stimulation or recording of action potentials); *F*, terminals for 'earth' connexion to the sea water in the lower part of the chamber, through a chlorided silver wire; *P*, inflow tube: the continuation of this tube to the 'nerve-tube' through the centre of the block of Perspex is indicated by the dotted lines. The nerve is held in the centre of the 'nerve-tube' by glass loops, *G*, which are fixed to the Perspex with cement. The bathing solution leaves the chamber through the out-flow tubes, *Q*. The tubes, *R*, at the top of the nerve chamber, fitted with rubber tubes and clips (not shown), are used for controlling the level of the solution in the chamber. In the side view the Perspex windows are removed, but the six screw holes for fastening are shown.

with one another, to minimize disturbances due to external mechanical movements, such as might be caused by a person walking across the room. The previous apparatus (Hill & Keynes, 1949) had its components mounted on different stands, and although these stands were massive and the table a very stout one, the slightest pressure of a finger on the table would give a very large movement of the galvanometer spot when working at high amplification. The present system was almost entirely free from this failing, being constructed in a very robust way, but to ensure even more complete freedom from disturbance it was mounted on a 3 ft. square slab of 1 in. slate, which itself rested on sorbo-rubber pads on the bench.

The shutter controlling the light entering the balancing photocell had to be capable of very fine adjustment. The system shown diagrammatically in Fig. 2 was built up from a reduction-gear unit, taken from an ex-R.A.F. bomb-sight. One turn of the controlling wheel moves the shutter 17 μ., and the minimum adjustment is about 1 μ. When working at the highest amplification this balancing adjustment has to be used to its finest limit when centring the galvanometer spot.

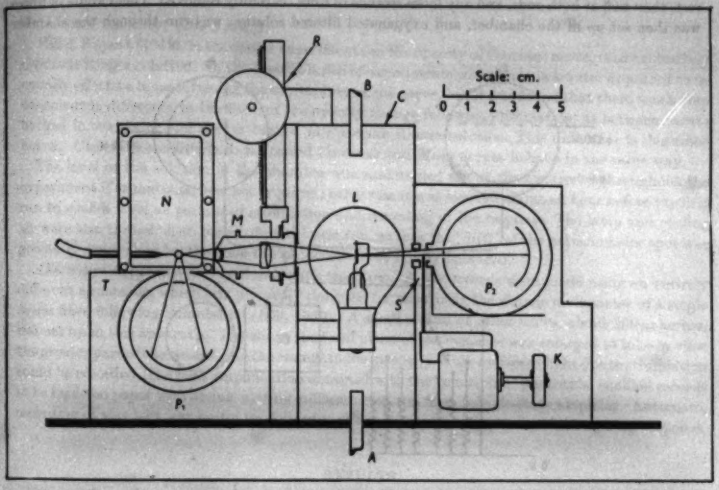

Fig. 2. General view of apparatus. *N*, nerve chamber; it is clamped down rigidly on the platform, *T*, by a device which is not shown in the diagram. The source of light, *L*, is a 36 W. 6 V. headlamp bulb in a metal housing, *C*, and is cooled by a stream of air entering by tube *A*, leaving by tube *B*. (The lamp filament is actually horizontal, and not vertical as indicated in the diagram.) A 2/3 in. microscope objective, *M*, focuses an image of the filament (about 0·5 mm. wide) on the nerve. The exact positioning of the filament image on the centre of the nerve is done by raising and lowering the objective by the rack and pinion, *R*; the rack is then firmly clamped by a screw. The light scattered by the nerve enters the photocell, P_1. The light entering the balancing photocell, P_2, is controlled by a shutter, *S*, mounted on a reduction gear unit which is controlled by the knob, *K*. One turn of this knob moves the shutter through a distance of 17 μ., and the minimum adjustment is about 1 μ. The recording instrument measures the *difference* in the photocurrents from P_1 and P_2, and initially this difference is made zero by the balancing control. The photocells are 'Cintel' V.A. 17 (vacuum).

It was found that the stability was not so good with the perfusion fluid running as it was with the latter turned off. It is not certain whether this was due to particles being swept through the beam of light, or to movement of the nerve caused by the stream.

Preparation of nerve. The limb nerve of the common spider-crab, *Maia squinado*, was used. It was shown that the effects with this nerve are essentially similar to those obtained with the nerve of *Carcinus maenas*, used in the earlier experiments by Hill & Keynes (1949). The nerve was prepared from the proximal link of one of the walking legs by the 'pulling out' method of Furusawa (1929). Cowan (1934) disapproved of this method, on the grounds that the resting potential of a nerve

isolated in this way was considerably lower than the potential recorded from a nerve prepared by cutting open the leg after the manner of Levin (1927). Although this would seem to indicate that the 'pulled out' nerve suffers some damage in the process, it was not found in the present experiments to differ from the nerve prepared by dissection, and survival for many hours testified to its good condition.

A compact bundle of fibres, generally about one-fifth part of the whole nerve, was freed from the rest, then tied at both ends, and any loose strands of fibres carefully removed. This bundle of fibres was then set up in the chamber, and oxygenated filtered solution was run through the chamber

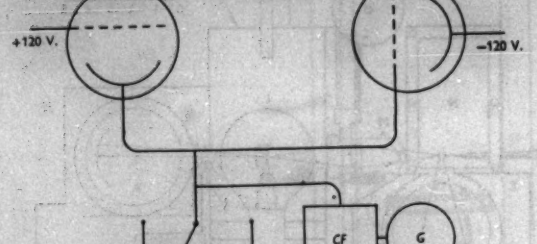

Fig. 3. The photocurrent amplifier circuit. The *difference* in the photocurrents from the recording cell, P_1, and the balancing cell, P_2, passes through one of the resistances, R, selected by a switch. The voltage developed across the resistance is measured by a cathode-follower unit, *CF*, which is similar to that described by Hill (1948), the final recording instrument being a Unicam 'Spot' galvanometer, *G*, resistance 1070 Ω., sensitivity 2 μA. for full-scale deflexion, and period 2·5 sec. The resistances, R, are 0·1, 1·0, 3·3, 10, 30, 60, 200 MΩ. (approximate values), and the ratios of amplification at each resistance to the amplification at 0·1 MΩ. are 1·0, 9·4, 33, 132, 409, 939, 2820 (measured values). The photocells are 'Cintel' V.A. 17 (vacuum).

continuously for 3–4 hr. to allow time for the nerve to attain equilibrium with its surroundings. The solutions used for bathing the nerve consisted either of sea water or of an artificially prepared *Carcinus* Ringer solution. In the experiments designed to investigate the effect of a change in external medium, the alternative solution was one of the above 'basic' solutions modified, either by dilution or by the addition of certain salts, as described later.

The composition of the sea water (calculated from Harvey's (1945) data) as regards major constituents, is as follows:

Na^+	471 mM./l.	Cl^-	549 mM./l.
K^+	9·9	$SO_4^=$	28·2
Ca^{++}	10·2	HCO_3^-	2·3
Mg^{++}	53·6		

Total ionic molarity, 1124·2 mM./l. Small variations in total molarity (or 'salinity') are to be expected from sample to sample.

The *Carcinus* Ringer solution was made according to Pantin's (1946) formula, and is based on Webb's (1940) analysis of *Carcinus* blood. Its ionic composition is as follows:

Na^+	493·6 mM./l.	Cl^-	535·2 mM./l.
K^+	11·3	SO_4^-	15·4
Ca^{++}	12·3	HCO_3^-	0·2
Mg^{++}	18·3		

Total ionic molarity, 1086·3 mM./l.

Hill & Keynes (1949), in the earlier experiments on the opacity of *Carcinus* nerve, used exclusively *Carcinus* Ringer solution. In the present series of experiments with *Maia*, sea water appeared to be equally effective in maintaining the excitability of the nerve, but it was found that there was a very conspicuous difference in the form of the opacity change following stimulation, as between nerves bathed in sea water and nerves bathed in *Carcinus* Ringer solution. This difference is described below. Under all conditions so far tested *Carcinus* and *Maia* nerves behave in the same way.

The level of the solution in the chamber was maintained above the electrodes throughout the experiment if stimulation was not required; otherwise it was lowered about an hour before the first run to such a level as permitted stimulation and recording of the response. The lamp and cooling air were also turned on an hour before the first run, so that the 'drift' of the galvanometer spot was generally quite slow by the time the photocells were finally balanced.

Alternative apparatus for rapid automatic recording. A few records were made using an entirely different apparatus, which was primarily designed for measuring the change in diameter of a single *Sepia* fibre following stimulation (Hill, 1950). A small strand of *Maia* nerve, about 200 μ. across, was set up in this apparatus. The slit in the focal plane of the eyepiece was enlarged to take in view the greater part of the strand, and the change in the intensity of the scattered light due to stimulation could be recorded. The high amplification obtained with the 'multiplier' phototube enabled records to be made on paper with an ink-writing milliammeter, run from a single-stage amplifier. Automatic recording of this sort was useful for following the more rapid early phases of the opacity response.

RESULTS

The effect of a change in the osmotic pressure of the external medium. The nerve is set up in the way described above, and one of the two normal solutions (i.e. sea water, or *Carcinus* Ringer solution) is passed through the chamber for at least an hour, until equilibration is complete. The second reservoir is filled with 'normal' solution to which has been added a known quantity of sodium chloride. The opacity of the nerve changes when this new solution is run in (Fig. 4). It is seen that an increase in 'strength' of the medium gives rise to an increase in the opacity. One result of the addition of solute to the external medium is to increase its refractive index, and the difference between the refractive indices of the medium and the nerve membrane is reduced. This, by itself, must result in a *decrease* in the scattering. In actual fact, the scattering *increases*. Another substance, magnesium sulphate, which is presumably also to be regarded as inert in respect of the resting nerve membrane, gives a similar result. It therefore seems reasonable to conclude that the increase in scattering is associated with, or accompanies, a decrease in fibre size, brought about by osmotic shrinkage. (The words: 'associated with, or accompanies' are intentionally used in this non-committal way, in order to avoid stating definitely that the effect is *due* to a change in fibre size, for there is no rigid proof that this is so.)

The refractive index is not, however, a negligible factor. If the added solute has a high molecular weight, the change in refractive index becomes relatively more important. In the case of sucrose the two opposing tendencies become nearly equal. The effect of an addition of sucrose has on one occasion been found to be as great as 40% of that due to an equi-osmotic increment of sodium chloride, but generally it is less, and on two occasions has been found to be almost zero. With magnesium sulphate there is less variability, but it is always found that the 'refractive index factor' reduces the 'osmotic effect' appreciably.

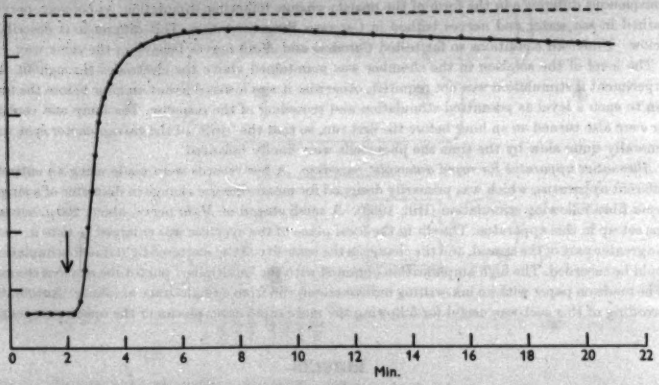

Fig. 4. The change in the intensity of light scattered by the nerve as the result of the addition of 26·8 mM. NaCl/l. to the medium. Normal medium, sea water. The switch from one reservoir to the other was made at a time indicated by the arrow.

As it is proposed to use the 'sodium chloride' standard for relating changes of scattering with changes in fibre diameter, it is necessary to estimate the extent to which refractive index is an important factor in this case. The molecular weight of sodium chloride is 58, of sucrose, 342. Taking the relative osmotic coefficients as 0·8:1·0, it follows that 58 g. of NaCl is equivalent, osmotically, to 615 g. of sucrose. The change of refractive index of an aqueous solution is nearly proportional to the weight of solute added, and the effects of sodium chloride and sucrose are practically the same, weight for weight. The 'refractive index factor' and the 'osmotic factor' are about equal for sucrose, so it follows that the effect with sodium chloride is reduced about 10% by the 'refractive index factor'. It will be seen later that the calculation of the apparent change in fibre diameter resulting from stimulation has necessarily to be rather inaccurate, and the above rather rough estimate of the importance of the refractive index is sufficiently precise for the purpose.

The kinetics of the penetration of water across the nerve membrane cannot be deduced from the form of the curve (such as that shown in Fig. 4), because the actual time course of the penetration of water is largely determined by the rate at which the new medium replaces the old between the fibres: the second fluid does not immediately replace the first in the nerve tube of the chamber (it is seen in Fig. 4 that there is a delay of about 30 sec. before the response even commences), and there is further delay due to the slowness of diffusion between the fibres. The rate of flow through the nerve chamber is purposely kept low to avoid movement of the nerve, and if the rate of flow is increased the attainment of the final equilibrium is hastened, for then the more rapid turbulent flow speeds up the complete exchange of fluid in the nerve tube.

The relation between scattering and osmotic pressure is approximately linear. This was shown by taking two solutions, one of which had an osmotic pressure 20% above, and the other 20% below, normal. The readings with these solutions, with normal solution as reference, were as follows:

Readings with normal solution as reference

Osmotic pressure 20% low		Osmotic pressure 20% high	
(2)	−238	(1)	+400
(4)	−338	(3)	+355
(6)	−354	(5)	+329
(8)	−349	(7)	+316
(10)	−311	(9)	+283

The runs were made in the order indicated by the numbers in brackets. At the start of the series, which took 3 hr. to complete, the effect of shrinkage exceeded the effect of swelling, but towards the end the position was reversed.

Solutes capable of penetrating the nerve membrane. If the solute added to the normal medium is capable of penetrating the nerve membrane the initial increase in scattering is followed by a regression as the penetration proceeds. This method has been used for studying the penetration of potassium chloride (Fig. 5) and urea (Fig. 6). The response to potassium chloride is dependent on the excess concentration. With a small addition, e.g. 9 mm./l., which about doubles the normal concentration, the regression to the base-line is more or less rapid, and considerable variability is found in the rate at which penetration occurs (Fig. 5). If a large excess of potassium is added, e.g. 50 mm./l., the initial 'osmotic' effect is very soon followed by a *decrease* in scattering, which is too large to be accounted for by the 'refractive index factor', and is probably to be attributed to the 'stimulating' action of potassium chloride in such concentration, akin to that produced by normal excitation (discussed below). The situation is complicated, because even in the absence of any 'stimulation', and basing a theoretical calculation simply on the assumption that a Donnan equilibrium governs the distribution of ions, the net volume change following the addition of potassium chloride cannot be estimated with any accuracy. This point is discussed later.

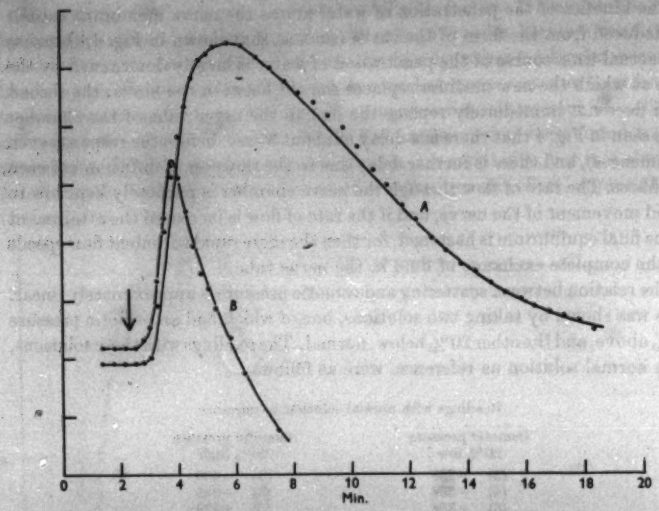

Fig. 5. The change in scattering following the addition of potassium chloride to the medium. Normal medium, sea water. *A*, addition of 9·1 mM. KCl/l.; *B*, addition of 53·6 mM. KCl/l. The switch from one reservoir to the other was made at a time indicated by the arrow.

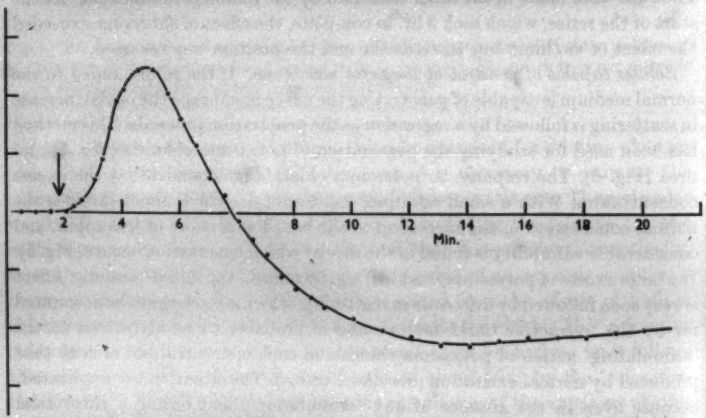

Fig. 6. The change in scattering following the addition of 66·6 mM. urea/l. to the medium (norma medium, sea water). The switch from one reservoir to the other was made at a time indicated by the arrow.

In the case of urea (Fig. 6), the return to a final level on the reverse side of the base-line can be attributed entirely to the 'refractive index factor', and does not indicate a concentration of urea by the nerve fibre.

The kinetics of penetration are to be deduced from the curves only when the substance in question penetrates very slowly. When the penetration is rapid, e.g. with urea, the time course is largely determined by the rate at which the new medium penetrates into the inter-fibre spaces.

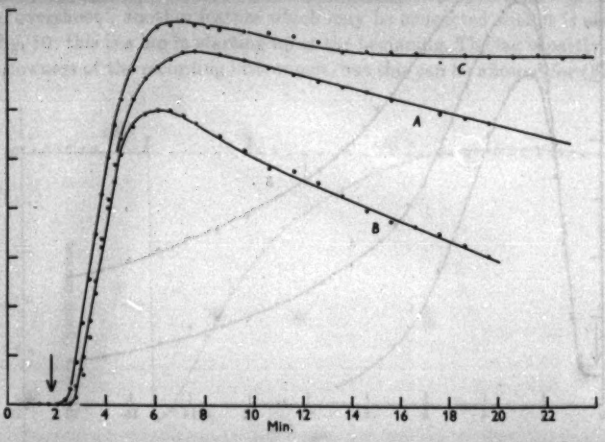

Fig. 7. The effect on the permeability to sodium chloride of altering the calcium concentration of the bathing solution. In each case 27·4 mM. NaCl/l. was added to the reference solution. *A*, reference solution was *Carcinus* Ringer solution with slightly subnormal calcium concentration (9·9 mM. Ca; normal is 12·3 mM. Ca). *B*, reference solution was Ca-free *Carcinus* Ringer solution. *C*, reference solution was *Carcinus* Ringer solution with additional calcium (total 52·7 mM. Ca). Runs made in the order *A*, *B*, *C*. Run *A* was taken 1 hr. after setting up the nerve in the chamber; *B*, after 3 hr. perfusion with Ca-free solution; *C*, after 2 hr. perfusion with the Ca-rich solution. The switch from one reservoir to the other was made at a time indicated by the arrow.

The effect of calcium on the permeability to sodium chloride. One instance of slow penetration, that of sodium chloride into the resting nerve, has been studied by this method. It will be seen from Fig. 4 that the osmotic shrinkage, following the addition of sodium chloride to the medium, shows a slow decline; this indicates, presumably, that sodium chloride is entering the fibre. This slow regression was always found to be present, but its rate was rather variable.

The effect of changing the calcium content of the medium is illustrated in Fig. 7. In a stream of calcium-free solution, three to four hours' soaking greatly increases the permeability. In this particular case (Fig. 7), the regression

with normal calcium concentration (10 mM./l.) was rather faster than it generally is. An increase in calcium well above the normal value brings about an almost complete stoppage of the regression. The slight initial decline from the peak in the high-calcium curve has been found in every experiment.

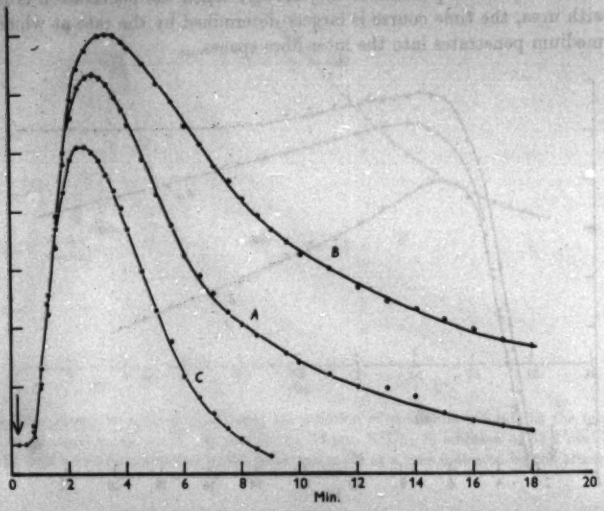

Fig. 8. The effect on the permeability to potassium chloride of altering the calcium concentration of the bathing solution. In each case 20 mM. KCl/l. was added to the reference solution. *A*, reference solution was normal sea water (10·2 mM. Ca); *B*, reference solution was sea water with additional calcium (total 65·2 mM.); *C*, reference solution was normal sea water. Runs made in the order *A*, *B*, *C*. Run *A* was made 1 hr. after setting up the nerve in the chamber; *B*, after 2½ hr. perfusion with Ca-rich solution; *C*, after further 2½ hr. perfusion with normal sea water. The switch from one reservoir to the other was made at a time indicated by the arrow.

The effect of calcium on the permeability to potassium chloride. The situation with potassium chloride (Fig. 8) is complicated by what has been referred to above as its 'stimulating' action. This gives rise to a swelling which, particularly at high concentrations, soon masks the osmotic effect (Fig. 5). A sign of this tendency is seen in curve *C* of Fig. 8, even though the potassium concentration was only three times its normal value. In calcium-free solution the initial shrinkage may be absent altogether. This 'de-sensitization' of the nerve by calcium makes the interpretation of the curves in Fig. 8 somewhat doubtful, although the conclusion that calcium decreases the passive permeability to potassium chloride would seem to be a reasonable one.

The effect of stimulation

The time course of the change in sea water

The early phase. When the nerve is soaked in sea water the opacity change due to stimulation at 50/sec. for 15 sec. is of the form shown in Fig. 9. The main response is a *decrease* in opacity. The maximum response does not coincide with the end of the period of stimulation, but it is delayed. With automatic recording on moving paper this initial stage is more clearly displayed (Fig. 10). Besides this 'overshoot', another feature which may be connected with it is also seen in Fig. 10: this is a lag in starting up at the beginning. The lag is partly due to the slowness of the recording instrument, but this can be allowed for (Fig. 10).

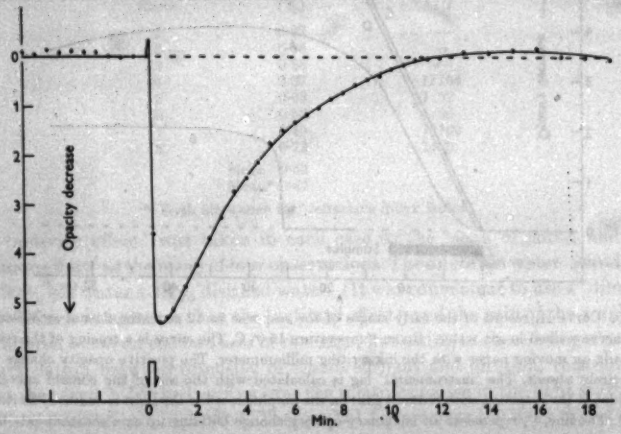

Fig. 9. The response to stimulation at 50/sec. for 15 sec. The stimulation period is indicated by arrows. *Maia* nerve in normal oxygenated sea water. Room temperature 18·5° C. Series resistance 60 MΩ. The ordinate scale is in arbitrary units. Base-line corrected for 'drift'. Recording by galvanometer; readings at 0·25, 0·5 or 1·0 min. intervals. An early positive opacity change is shown, but it could not be recorded accurately.

The recovery phase. Fig. 9 shows a prolonged recovery following the initial peak. In the earlier paper, Hill & Keynes (1949) were unable to follow this phase with any regularity, but it now appears that it follows about the same time course as the recovery heat production of *Maia* nerve. A. V. Hill (1929, p. 165) gives the heat production in 30 sec. intervals following a 6 sec. stimulus of *Maia* nerve at 16·8° C. Ignoring the heat production in the first 30 sec. interval (to avoid confusion with the 'initial heat'), half of the remaining heat is produced by 5·5 min. after the beginning of the stimulus. By comparison, the curve of Fig. 9 has returned from the peak half-way to the base-line in

3·7 min. (18·5° C.). The run selected for Fig. 9 is not entirely representative of the sixteen which have been recorded under similar conditions on other nerves, because in most instances the curve either did not completely return to the base-line, or it overshot, and there was a tendency for the drift rate of the base-line to undergo a change. However, the curves in their general form were not greatly different from the one in Fig. 9, but the 'half times' of recovery are not sufficiently well defined to make it worth pursuing the comparison with heat production. The recovery phase has also been recorded with solution flowing

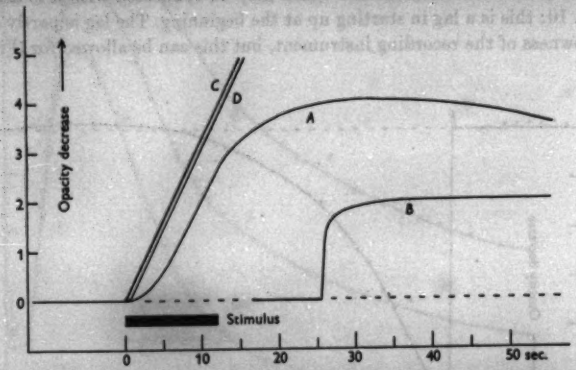

Fig. 10. Curve *A*, record of the early stages of the response to 12 sec. stimulation at 50/sec. of a nerve soaked in sea water. Room temperature 15·0° C. The curve is a tracing of the record made on moving paper with the ink-writing milliammeter. The positive opacity change was entirely absent. The 'instrumental' lag is calculated with the aid of the control curve, *B*, which is the response, with reduced gain on the amplifier, when the light is suddenly switched off. The line, *C*, represents an imaginary opacity change building up at a constant rate from time zero, and recorded without lag. The instrumental lag would cause a delay in recording, and the actual response would be as indicated by *D*. It is seen that there is a further, non-instrumental, lag in the response of the nerve to stimulation.

continuously through the chamber. This was done because it was thought possible that the temperature in the nerve tube might be considerably elevated owing to the intense light, and that the recovery might consequently be speeded up. However, the curve was not perceptibly affected.

Comparison of the effect of stimulation with a change in external osmotic pressure. In the argument to correlate the effect of stimulation with changes in fibre size, there seems to be no possibility of deriving a theoretical relation, on the basis of geometrical optics, between the intensity of scattered light and the fibre size. In any case it may not necessarily be true that the factor primarily determining the opacity is the size *per se*, for another possible factor is the degree of dilution of the internal colloidal particles. If this were the operative factor, there is even

less hope of calculating the relation between size and opacity, because the required relation between opacity and dilution is not known. However, without prejudging the real nature of the effect, one may experimentally compare the change due to activity with the effect of an approximately known change in fibre size brought about by an alteration in external osmotic pressure. As a measure of the 'activity' effect one takes the peak value of the response (Fig. 9), and compares this effect with a known dilution of the sea water surrounding the nerve. This was done for eight different nerves, each stimulated at 50 impulses/sec. for 10 sec. (500 impulses). Allowance must be made for the 'refractive index factor', taken as for sodium chloride. The results were as follows:

Nerve	Equivalent dilution (%)	$\Delta I/I$
1	0·62	—
2	0·58	—
3	0·28	—
4	0·37	1:164
5	0·63	1:92
6	0·52	1:90
7	0·45	1:109
8	0·73	1:79
	Mean 0·52	
	Mean* 0·47	

* With allowance for 'refractive index factor'.

The 'activity effect' was taken in each case as the mean of three, and the 'dilution effect' as the mean of four observations. The dilute sea water 'standard' was 90% sea water + 10% distilled water. (It was convenient to use a 'dilution effect' which was greater than the 'activity effect', and the gain of the amplifier was altered between the two sets of readings, but owing to there being a nearly linear relation between intensity of scattered light and dilution, this did not affect the result.) In the last five runs the relative change in the light scattered by the nerve was also measured. The symbol I represents the intensity of light scattered by the unstimulated nerve, and ΔI the peak change due to 500 impulses, and the ratios $\Delta I/I$ for these five runs are included in the above table. The measurement of the intensity I involved the removal of the nerve from the chamber, in order to make allowance for the light scattered into the photocell by the chamber itself.

The ratio $\Delta I/I$ can be slightly increased by using blue light, obtained by placing a colour filter in the light beam. The advantage gained by increasing the ratio was, however, more than offset by having to work with a greatly reduced light intensity.

The time course of the change in Carcinus *Ringer solution or in diluted sea water*

The records made by Hill & Keynes (1949) showed a large initial *increase* in opacity following stimulation. Subsequently, when further experiments on similar lines were done with the modified apparatus described here, it was

found that this early phase of *increase* was either relatively small (as in Fig. 9) or was often completely missing (as in Fig. 10), the response being simply a decrease in opacity. *Carcinus* and *Maia* nerves behaved similarly in this respect. This difference was found to be due to the fact that in the earlier experiments the nerve was soaked in *Carcinus* Ringer solution, while in the later ones sea water was used (the nerve survived equally well in either solution). The transition from one type of response to the other could be made simply by changing

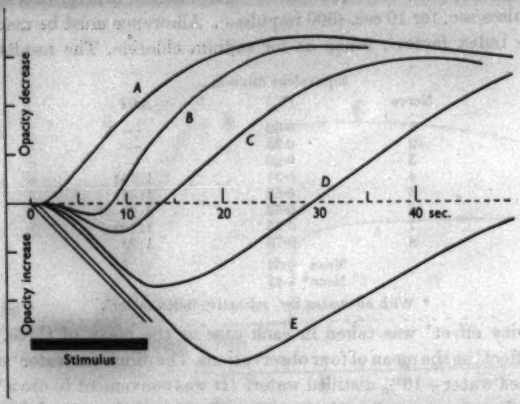

Fig. 11. The response to stimulation in dilute sea water, or in *Carcinus* Ringer solution. The curves are not tracings of actual records, because a series as complete as this has not been recorded on any one nerve. Curve *E* approximately represents the response to 12 sec. stimulation at 50/sec. of a nerve transferred to 80 % sea water 10 min. earlier. The two lines to the left of curve *E* have the same significance as those shown in Fig. 10, and illustrate the lag in commencement of the response. Curve *D* is typical of records made with 90–95 % sea water or in *Carcinus* Ringer solution. Curves *B* and *C* are types which may be observed with the nerve in normal sea water; or if the normal response does not commence with a positive phase (curve *A*), some dilution is required to bring out the forms *B* or *C*. At any given dilution there is considerable variability, as between different nerves, in the relative proportions of the positive and negative phases, and in the time at which the curve crosses the base-line.

the solution around the nerve: after about 5 min. pause the nerve could be stimulated and the transition found to be complete. At first it was thought that this must depend on the difference in the relative proportions of the various ions in the two solutions, but by making suitable tests this was found not to be the explanation. It turned out that a difference in total concentration (or osmotic pressure) was alone responsible. An initial positive phase could be made to appear simply by diluting the sea water surrounding the nerve, and the relative size of this phase, and the time at which it reversed were dependent on the degree of dilution (Fig. 11). To confirm that the effects with sea water

and *Carcinus* Ringer solution differ for this same reason, the osmotic pressures were compared by measuring the change in opacity of the nerve when one solution was replaced by the other, and by standardizing this difference against a known dilution of sea water. It was found that the osmotic pressure of *Carcinus* Ringer solution is equal to that of 95·1% sea water + 4·9% distilled water. A 5% dilution of the sea water bathing the nerve caused the appearance of the positive phase, and it was then relatively about as large as it is with the same nerve in *Carcinus* Ringer solution.

The effect of *increasing* the osmotic pressure of the solution by 10% above the normal value for sea water was a reduction in the size of the negative opacity phase. This is the reverse of what might be expected. The test was made by comparing the peak values of the negative opacity changes following 50/sec. stimulation for 10 sec. in normal sea water and in sea water enriched with 3·0 g./l. sodium chloride, giving a difference in osmotic pressure of about 10%. Observations were taken in the following order:

110% sea water, three runs, peak values 19, 20, 21 units;
100% sea water, two runs, peak values 46, 37 units;
110% sea water, three runs, peak values 20, 25, 25 units;
100% sea water, three runs, peak values 35, 37, 35 units.

It is clear from these figures that the response is reduced by increasing the external osmotic pressure.

The question which now arises is this: which type of response must be considered as representing the 'normal' function? The change from the one form to the other is brought about by a remarkably small change in the conditions, for example, a 3 or 4% dilution may cause the appearance of a large positive phase where none existed before. Although *Carcinus* Ringer solution is more like crustacean blood than is sea water as regards the relative proportions of the different ions, there is no good evidence that, to within 3 or 4%, the osmotic pressure of *Carcinus* Ringer solution is more nearly equal to that of blood. The method which has been used in the attempt to decide this point involves the possibly questionable assumption that if the nerve is equilibrated for long enough in any one of these media the response should tend to become 'normal'. Making use of this criterion, it appears that a large early positive phase is the abnormal response. This was tested by soaking nerves for long periods in *Carcinus* Ringer solution or in diluted sea water. In either case the early positive phase, which was large at the start, diminished steadily, and at the end of 3–4 hr. soaking the response was practically the same as that of a 'control' nerve in normal sea water: the latter hardly altered with time. On the other hand, it is not so certain whether a *small* early positive phase should be considered as abnormal. In some instances it has persisted up to 4–5 hr. in sea water—as in the case shown in Fig. 9—but often it was neither seen at the start, nor did it appear later.

The time course of the positive phase, recorded when it is most pronounced shortly after dilution or the application of *Carcinus* Ringer solution, shows that there is a lag in starting off (Fig. 11). This lag is about the same as was found for the commencement of the negative phase for a nerve in normal sea water (Fig. 10).

DISCUSSION

Stimulation of a nerve trunk has been seen to produce a decrease in opacity, and this may, in certain circumstances, be preceded by a phase of increased opacity. Concentrating attention in the first place on the *decrease*, which is invariably shown, evidence has been produced which suggests that this is due to an increase in diameter of the fibres, for when the fibres are made to swell by diluting the external medium, the opacity decreases. Hill (1950) has shown that a single giant fibre from the cuttlefish swells when it is stimulated, and there are good reasons why it should do so. The effect of stimulation is to increase the internal osmotic pressure of a nerve fibre, and this results in swelling due to the inward passage of water. The osmotic pressure increases for the following reasons: (*a*) stimulation causes an exchange of sodium and potassium across the nerve membrane, sodium entering and potassium leaving the fibre. Potassium is osmotically less active than sodium, and the internal osmotic pressure therefore increases; (*b*) in addition to the exchange of sodium and potassium, some sodium enters the fibre accompanied by chloride ions. In the first part of the discussion an attempt is made to correlate the opacity change with the movement of ions.

The second part of the discussion turns on the nature of the initial *increase* of opacity which may appear in some circumstances. This increase suggests a decrease in fibre size, but the possible reasons for a shrinkage are not so clear (Hill, 1950), and the discussion becomes rather more speculative.

Lastly, the question of the permeability of the resting fibre to sodium ions and to other substances is touched on.

It must be stressed that no proof can be advanced of the tacit assumption that the changes in opacity as observed are solely due to alterations in fibre size and not partly due to changes, independent of size, in opacity of the axoplasm or membrane of the individual fibre. Even if the effects *are* due to a swelling or shrinkage of the fibres, it is by no means plain why the opacity changes as it does. It was pointed out earlier that at present there is no criterion which enables one to decide whether the size *per se* is responsible, or whether the associated change in the degree of dilution of the internal colloidal particles is the operative factor.

The decrease in opacity following stimulation

The effect of 500 impulses has been found to produce a decrease in opacity equal to that brought about by diluting the external medium by 0·47%. To

translate this figure into the corresponding actual change of diameter it is necessary to select an 'equivalent diameter' to represent the fibres as a whole. Young (1936) states that the axons of the leg nerves of *Maia* vary in diameter from 'less than 1 μ. to 20 μ.'. The majority of the fibres are at the small end of this range, and as it seems probable that the opacity of a nerve depends to a greater degree on the small fibres than on the large ones, the upper and lower limits of the 'equivalent diameter' are taken as 0·5 and 5 μ. In calculating the corresponding increase of diameter for 0·47 % dilution, allowance has to be made for a fraction of the volume of the fibre being osmotically inactive. Taking this fraction as one-quarter (estimated on the basis of evidence presented by Lucké, 1940) the increase in fibre diameter produced by 500 impulses is 0·18 %, and therefore lies between 0·0009 and 0·009 μ. Is this the order of magnitude one might expect? The extra potassium lost during stimulation from whole *Maia* nerve has been measured by Cowan (1934); he gives the figure as $2{\cdot}92 \times 10^{-10}$ mole K/g./impulse. Young's (1936) histological data suggest that there are roughly 2000 sq.cm. of membrane in 1 g. of nerve. This gives a figure of $0{\cdot}15 \times 10^{-12}$ mole K/cm.^{2}./impulse. Keynes (1949) finds about twice this value for the leakage of potassium from a stimulated *Carcinus* nerve. Cowan's nerves were 'stimulated to fatigue' and many axons may have become inexcitable before the end; it is also possible that considerable quantities of potassium may have been absorbed from the interstitial spaces of the nerve trunk during the 5 min. period of stimulation employed. Keynes, in referring to his results, remarks that the 'action potential always declined, often to half or one-third of its original size during the course of the experiment'. A figure rather greater than $0{\cdot}3 \times 10^{-12}$ mole K/cm.^{2}/impulse should therefore be taken. In the single fibre of *Sepia* the outflow of potassium is $4{\cdot}5 \times 10^{-12}$ mole K/cm.^{2}/impulse (Keynes, 1950), and in the smaller single fibres of *Carcinus* it is $1{\cdot}7 \times 10^{-12}$ mole K/cm.^{2}/impulse (Hodgkin, 1947). A value of $1{\cdot}0 \times 10^{-12}$ mole K/cm.^{2}/impulse cannot be far from the true value for the leakage of potassium in whole *Maia* nerve. What change of osmotic pressure and increase of fibre diameter results from an exchange of this quantity of potassium with an equivalent quantity of sodium? It is shown (Hill, 1950) that the exchange of one ion of sodium with one of potassium results in the entry into the fibre of 3·2 molecules of water. The exchange of $1{\cdot}0 \times 10^{-12}$ mole K/cm.^{2}/impulse will, owing to this entry of water, result in an increase in fibre diameter of $1{\cdot}1 \times 10^{-6}$ μ./impulse. For 500 impulses this is 0·0006 μ., which is less than the minimum 0·0009 μ. estimated to occur. The difference, 0·0003 μ., may be assumed to be made up by the entry of sodium chloride (Hill, 1950). It can be shown that an entry of $3{\cdot}3 \times 10^{-14}$ mole NaCl/cm.^{2}/impulse is sufficient to give an increase of 0·0003 μ./500 impulses. This ionic *transfer* amounts to about 3·3 % of the ionic *exchange*, and the amount of sodium entering the fibre during activity is 3·3 % greater than the amount of potassium leaving, the excess of sodium being accompanied

by chloride. If one takes the upper limit of 0·009 μ. for the actual change, the difference is 0·0084 μ., and an entry of $9{\cdot}2 \times 10^{-13}$ mole NaCl/cm.^{2}/impulse is required. In this case the transfer amounts to 92% of the exchange.

Hill (1950) estimated that the transfer of sodium in a stimulated *Sepia* fibre was about 38% of the exchange.

A direct analytical test of the relative quantities of sodium entering and potassium leaving the fibre during activity would help to narrow down the range of possibilities, but until this is available the potassium outflow must be employed as a guide to the whole ionic exchange. It appears, however, that the orders of magnitude are right, and the phase of decreased opacity is therefore attributable to swelling of the individual nerve fibres. The increase in diameter appears to lie between 0·0009 and 0·009 μ. for 500 impulses.

The initial increase of opacity

It has been found that dilution of the solution around the nerve has a striking effect on the opacity change following stimulation, for a large initial *increase* in opacity is found then to precede the main decrease. A small early positive phase is often seen with a nerve in normal solution, but it is sometimes entirely absent. An increase in opacity indicates a decrease in fibre size.

It has been shown that a single *Sepia* fibre undergoes a very small shrinkage before the main swelling (Hill, 1950). The only explanation which could be offered was this: that in the rapid exchange of sodium and potassium, the potassium remains associated with its hydration water, which accompanies the ion through the membrane, and the sodium ion is forced to part with its hydration water, the latter following slowly afterwards under normal osmotic forces to replace that taken up by the sodium ion when it reaches the other side. Conway (1947) gives sodium as being hydrated with 8·0 molecules of water, and potassium 3·8 molecules. If sodium is entirely stripped of water and potassium not at all, the decrease in diameter of a nerve fibre would be 0·0007 μ. This is therefore a possible explanation, because 0·0007 μ. is comparable with the actual change and, although it may be more or less masked by the supervention of the phase of decreased opacity, a change of this order of magnitude might be observable. However, if this is the explanation it is not plain why the early positive phase should be so greatly exaggerated by dilution of the medium. For what may be called the 'dilution effect' another explanation must be sought. The only suggestion which seems at all plausible is that the hydrostatic pressure inside a swollen fibre is in some way responsible for the phenomenon. In the unstimulated fibre, the membrane of which is almost impermeable to sodium ions, dilution must result in a swelling which is permanent, or which regresses only slowly, for the conditions required for the Donnan equilibrium have to be fulfilled, and equality maintained in the activity products of the internal and external potassium and chloride ions. If, however, the perme-

ability to sodium is suddenly increased to a large value, as by stimulation, the Donnan equation no longer applies, sodium and chloride enter the fibre, potassium leaves, and the fibre swells: this has been discussed earlier. But if the interior of the fibre is initially under hydrostatic pressure due to a stretched membrane, there will be a tendency for the fibre to shrink, which could come about in the active fibre by a rapid extrusion of potassium and chloride ions. In this case water would pass out *with* the ions, whereas the water *follows* the ions if they move under concentration gradients. Thus the net ionic exchange may be inwards right from the start, and yet the form of the response be diphasic.

Perhaps the hydrostatic pressure in a fibre under normal conditions is appreciable, so the same explanation might be offered for the small initial positive phase seen with a nerve in a normal medium, and for the initial shrinkage of a *Sepia* fibre.

The permeability of the resting fibre to sodium ions and other substances

It has been shown that an increase of the osmotic pressure of the external medium has the immediate effect of causing a decrease in volume of the fibres. But this is not permanent: there tends to be a regression to the original fibre size. If the change in osmotic pressure is brought about by the addition of urea or potassium chloride this regression is easily accounted for: urea simply penetrates the fibre until its internal and external concentrations are the same, so the volume returns to its normal value; in the case of potassium chloride the controlling force is probably the unbalance in the products of the potassium and chloride activities, $[K] \times [Cl]$, inside and outside the fibre. This results in the entry of sodium chloride.

It has been shown by Boyle & Conway (1941) that there will be a return to exactly the original volume only if the potassium activity within the normal fibre is exactly one-half of the total internal ionic activity; although it appears that this condition obtains for the frog's sartorius muscle, the evidence (Shanes, 1946) that it does so for nerve is rather incomplete, and the volume of a nerve following the addition of potassium chloride to the medium should not necessarily be expected to return exactly to its original value.

There is some difficulty, however, in accounting for the slow reversal of the immediate change in volume due to the addition of sodium chloride. The immediate change, due to the passage of water, restores the osmotic balance across the membrane. The slight unbalance in the $[K] \times [Cl]$ ratio associated with this will contribute a further, but relatively small, decrease of volume; but it is not plain what forces produce the reversal, which in a calcium-free medium appears to restore the volume almost to its original value. One possible explanation is as follows: the permeability of the nerve membrane to sodium is not zero, but the inward leakage of sodium must normally be reversed by some active extrusion process. If, for some reason, the rate of entry of sodium is increased to such an extent that the extrusion process cannot cope with the

leakage, sodium chloride will enter the fibre. It is known that an isolated nerve in sea water, or other artificial medium, loses potassium and gains sodium, until it finally gets into a steady state: the rate of entry is then presumably just low enough to be reversed by the extrusion mechanism. A nerve in this condition should react to addition of sodium chloride in just the way that is seen; that is to say, the immediate response will be a shrinkage to restore osmotic balance: this will be followed by a regression, because the increased rate of entry of sodium brought about by the raised external concentration is greater than the extrusion process can deal with. The net intake of sodium ions is electrically balanced by an outflow of potassium ions, but also by an accompanying inward flow of chloride ions. It has been pointed out earlier that both of these processes result in swelling of the fibre. Lack of calcium increases the rate of regression, which seems to indicate that it raises the permeability to sodium, and an excess of calcium decreases it; or, alternatively, the rate of extrusion is affected. The complete stoppage of the regression in a solution containing five times the normal calcium concentration, suggests that the fibre is then in a condition such that the extrusion mechanism is capable of expelling sodium at a rate greater than the rate at which it enters in normal sea water; consequently, when the external concentration is raised it is still able to expel the total quantity which enters, and there is no net transfer. No explanation can be offered for the initial fall of the curve from the peak in high-calcium solution (Fig. 7).

The swelling due to dilution would be expected to regress slowly with time, owing to the *reduced* inflow of sodium, but this has not been tested.

SUMMARY

1. The change in the opacity to white light of a crustacean nerve trunk following repetitive stimulation has been further investigated. The effect is measured by making photoelectric recordings of the intensity of light scattered by the nerve at right angles to the incident beam.

2. The chamber for holding the nerve was designed so that, in addition to recording the effect of stimulation, the change in opacity brought about by alterations in the composition of the solution surrounding the nerve could also be investigated. It was found that the opacity of the nerve is very sensitive to changes in fibre diameter, brought about by altering the osmotic pressure of the solution. When the fibres swell the opacity decreases, when they shrink it increases.

3. The response to stimulation at 50–100/sec. in normal sea water consists of an initial small increase of opacity, which is rather variable and may be absent, followed by a larger decrease, which reaches its maximum shortly after the end of the 5–10 sec. period of stimulation, and then reverses slowly. At its peak the decrease in the intensity of the scattered light, due to stimulation at

50/sec. for 10 sec., is about 1 part in 100 of the resting intensity. Recovery appears to be complete in about 10–15 min. It is shown that this latter phase of decreased opacity can probably be attributed to an increase in diameter of the individual nerve fibres, and it is calculated that this increase lies between 0·0009 and 0·009 μ. for 500 impulses.

4. The initial increase of opacity due to stimulation can be greatly exaggerated by slight dilution of the solution surrounding the nerve. The reason for this is not clear.

5. The dependence of the opacity upon the fibre size has been made use of in studying the permeability of the fibre membrane to certain solutes. There is evidence which suggests that the permeability to sodium ions can be increased by removing calcium from the solution, and decreased by adding an excess.

REFERENCES

Boyle, P. J. & Conway, E. J. (1941). *J. Physiol.* **100**, 1.

Conway, E. J. (1947). *Irish. J. Med. Sci.* (Oct.–Nov.).

Cowan, S. L. (1934). *Proc. Roy. Soc.* B, **115**, 216.

Furusawa, K. (1929). *J. Physiol.* **67**, 325.

Harvey, H. W. (1945). *Recent Advances in Chemistry and Biology of Sea water.* Cambridge University Press.

Hill, A. V. (1929). *Proc. Roy. Soc.* B, **105**, 153.

Hill, D. K. (1948). *J. Physiol.* **107**, 479.

Hill, D. K. (1950). *J. Physiol.* **111**, 304.

Hill, D. K. & Keynes, R. D. (1949). *J. Physiol.* **108**, 278.

Hodgkin, A. L. (1947). *J. Physiol.* **106**, 341.

Keynes, R. D. (1949). Ph.D. Thesis. Cambridge University.

Keynes, R. D. (1950). *J. Physiol.* (in the Press).

Levin, A. (1927). *J. Physiol.* **63**, 113.

Lucké, B. (1940). *Symp. quant. Biol.* **8**, 123.

Pantin, C. F. A. (1946). *Microscopical Techniques for Zoologists.* Cambridge University Press.

Shanes, A. M. (1946). *J. cell. comp. Physiol.* **27**, 115.

Webb, D. A. (1940). *Proc. Roy. Soc.* B, **129**, 107.

Young, J. Z. (1936). *Proc. Roy. Soc.* B, **121**, 319.

J. Physiol. (1950) 111, 304–327 612.014.462.1.081

THE VOLUME CHANGE RESULTING FROM STIMULATION OF A GIANT NERVE FIBRE

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It has been shown (Hill & Keynes, 1949) that there is a change in the opacity of a crustacean nerve trunk when it is stimulated at high frequency for several seconds. The cause of this phenomenon was at first obscure, but further experiments (Hill, 1950) have suggested that it is due to a change in volume of the fibres. From the facts which have been inferred about ionic transfer and exchange in the active nerve fibre (Hodgkin & Huxley, 1947; Hodgkin & Katz, 1949; Keynes, 1950), stimulation would be expected to result in an extremely small increase in fibre volume. On this basis it was argued by Hill (1950) that 500 impulses might be expected to cause the diameter of individual fibres of a crustacean nerve to increase by 0·0009–0·009 μ., and there was evidence that a change of this order of magnitude could give rise to the observed change in opacity. However, the conclusion was not founded on any direct proof that such a change actually takes place, for there are other possible causes of a change in opacity. The single fibre preparation of the cuttlefish (*Sepia officinalis*) offered a means of testing the hypothesis, and this paper describes how it has been possible to record changes in diameter of this giant fibre when it is stimulated.

In certain circumstances the fibres of a nerve trunk appear to shrink before swelling (Hill, 1950). It will be seen that an initial shrinkage takes place also in the single *Sepia* fibre.

METHOD

The method of dissecting the single *Sepia* fibre is similar to that used in preparing the giant fibre of *Loligo* (see Young (1936), for a description of the nervous system of *Sepia officinalis*; and Cole & Curtis (1939), for the method of dissecting the giant axon of the squid, *Loligo pealii*). The diameter of the fibre was generally between 180 and 250 μ. (unstretched), although two unusually large specimens had fibres of 270 and 390 μ. The fibre was ligatured at both ends, to prevent exudation of axoplasm. The length required for the nerve chamber was 35 mm., which is easily obtained, even from the smaller specimens. Although the *Sepia* axon is less than half the size of the *Loligo* axon, the dissection of a single fibre is considered to be rather easier; it is facilitated by absence of side branches from the main axon which, in *Loligo*, increase the difficulty in the final stages of the dissection. Another difference from *Loligo* is that in *Sepia* the largest fibre is not unique, but there

are several, perhaps up to ten, equally large fibres supplying the inferior part of the mantle muscle. This richer supply of trunk nerves would naturally explain the absence of side branches.

Clean sea water, collected some miles off-shore, was used as a medium for the nerve.

The fibre was mounted in the chamber shown in Fig. 1*a*, being held in position by the electrodes. The chamber was then placed in position, in oxygenated sea water, on the flat-bottomed dish over the substage of the microscope. The fibre was illuminated from below (Fig. 1*b*) with a high-power dark-ground condenser of the cardioid type, using immersion oil between the upper lens of the

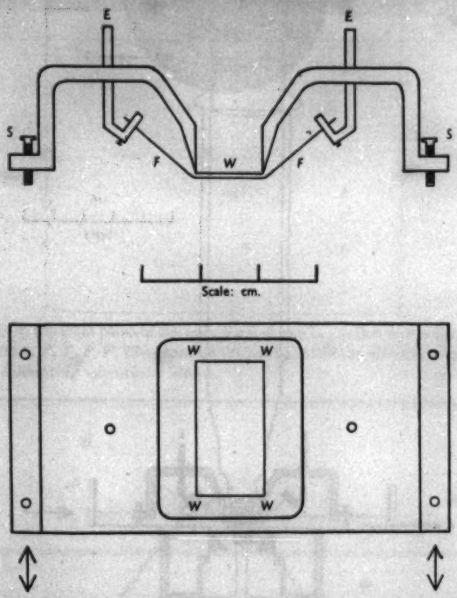

Fig. 1*a*. Diagram of chamber for holding nerve fibre, in vertical section (above) and plan view (below). *F*, *F*, nerve fibre; *E*, *E*, electrodes for stimulating and recording; *S*, *S*, screws for adjustment of clearance from bottom of trough. The double arrows by the plan view indicate the direction in which the nerve chamber is moved by the micrometer screw. *W*, *W*, *W*, *W* (plan view) indicates the edge of the cover-slip window, shown also as *W* (section view). It is sealed with cement to the main body of the chamber, which is constructed of Perspex.

condenser and the flat bottom of the dish. The condenser was exactly alined with a $\frac{1}{4}$ in. objective mounted in the microscope tube. This objective had to be rather narrow to fit into the space available in the nerve chamber, and was made up specially from the component lenses of another, more bulky, objective which was broken down for the purpose. The nerve chamber could be moved bodily in a horizontal direction, at right angles to the fibre axis, by means of a micrometer screw graduated in divisions of 2μ. The purpose of this device was for calibration, as described below. A circular glass disk in the focal plane of the eyepiece was blacked out except for a slit across the centre (Fig. 2). The slit was parallel to the fibre: when used with the $\frac{1}{4}$ in. objective it had an effective width of about 15μ. A 'multiplier' phototube (RCA 931 A., run at 860 V.) collected the light passing

up through the slit. The dark-ground condenser was adjusted in such a way that the edge of the fibre could be brightly illuminated, and appeared as an intense band of light. The 'background'

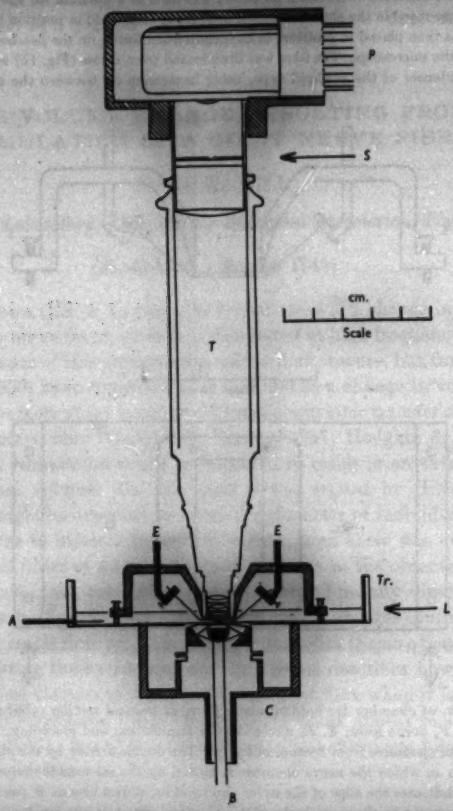

Fig. 1*b*. The nerve chamber, shown separately in Fig. 1*a*, is in position on the trough, *Tr*. *L* indicates the level of the sea water in the trough. The sea water is at earth potential through the Ag-AgCl electrode, *A*. The edge of the nerve fibre is illuminated obliquely by means of the dark-field condenser, *C*. *B* shows the beam of light from a 6 V. 24 W. tungsten filament lamp entering the condenser. The light scattered from the edge of the fibre passes up the microscope tube, *T*, and is partly intercepted by the slit, *S*, in the focal plane of the eyepiece. The light transmitted by the slit enters the phototube, *P*.

and the main bulk of the fibre were both relatively dark. The width of this bright band was about 10 μ., and was rather narrower than the width of the slit. The calibration curve (Fig. 3), relating light intensity (as measured at the photocell) with movement of the micrometer screw, was recorded

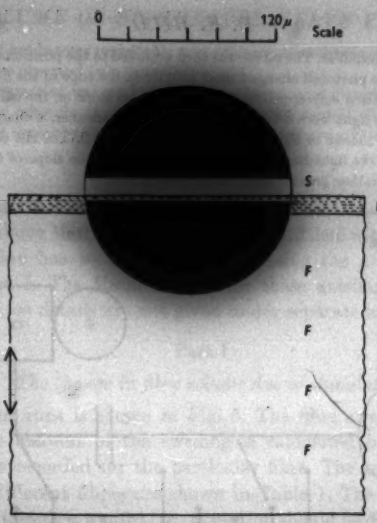

Fig. 2. The slit, *S*, in the focal plane of the eyepiece is shown in relation to the illuminated edge, *E*, of the nerve fibre, *F*, *F*, *F*, *F*. The arrow on the left indicates the direction of movement of the fibre by the calibrating micrometer screw.

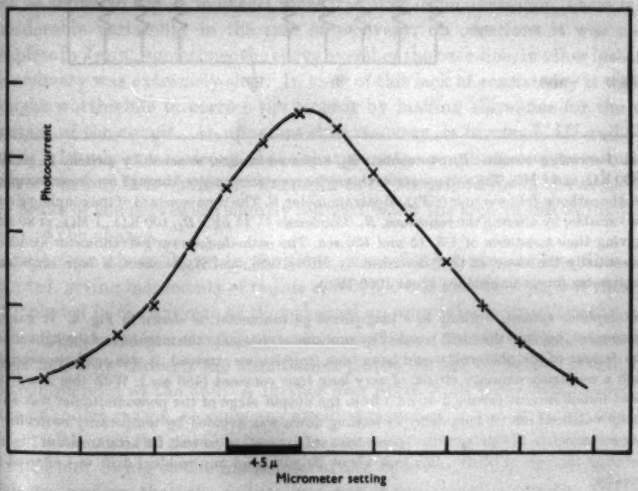

Fig. 3. Sample calibration curve. The photocurrent is shown in arbitrary units. Readings are indicated by crosses, ×.

with a low gain on the amplifier. The screw was then adjusted to the position at which the intensity of the light entering the photocell changed most rapidly as the edge of the fibre was moved; or, in other words, to the position corresponding to the maximum slope on the calibration curve. When the slit and the band of light were set in this relation to one another, a change in diameter of the fibre gave a maximum change in light intensity at the photocell. The slit edge was generally set upon the outer edge of the light band, because in this position the slope of the intensity-distance curve was found to be rather greater than on the inside.

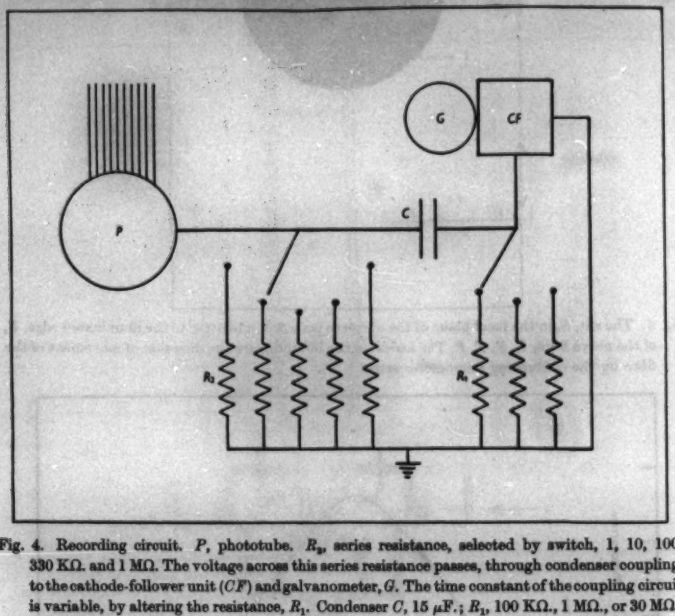

Fig. 4. Recording circuit. P, phototube. R_2, series resistance, selected by switch, 1, 10, 100, 330 KΩ. and 1 MΩ. The voltage across this series resistance passes, through condenser coupling, to the cathode-follower unit (CF) and galvanometer, G. The time constant of the coupling circuit is variable, by altering the resistance, R_1. Condenser C, 15 μF.; R_1, 100 KΩ., 1 MΩ., or 30 MΩ., giving time constants of 1·5, 15 and 450 sec. The cathode-follower-galvanometer system is essentially the same as that described by Hill (1948), and is, in effect, a high impedance voltmeter (input impedance about 1000 MΩ.).

The amplifier system, working to a long-period galvanometer, is shown in Fig. 4. It was not direct-coupled, because the 'drift' caused by continuous change in the intensity of the light source and by fatigue of the photocell would have been troublesome; instead, it was condenser-coupled through a resistance-capacity circuit of very long time constant (450 sec.). With this circuit the constant initial current (about 2–10 μA.) from the output stage of the photomultiplier was automatically balanced off. A long delay in settling down was avoided by temporarily switching to a lower resistance in the RC circuit. It was then only necessary to wait for attainment of the bias representing the rate of 'drift'; this took about 20 min., and any residual drift was allowed for graphically.

The nerve was stimulated at 100 or 200/sec. by a multi-vibrator unit, generally for 1 min., and the galvanometer readings taken at 15 sec. intervals before, during and after stimulation. A fibre

in good condition did not become inexcitable even after ten or twenty such periods of stimulation. Action potentials were observed on a cathode-ray tube.

The limit to discrimination was set by the random background disturbance ('noise') from the photocell, and not by other forms of disturbance such as mechanical vibration. The 'noise' was smoothed out to some extent by over-damping the galvanometer, to give it a period of about 5 sec.

RESULTS

The following section is divided into three parts. The first describes the results obtained by the above Method; the second and third deal with ancillary matters, to which attention has to be given in discussing the nature of the effects described in Part I. The Methods used for these ancillary experiments are relatively simple, so details are not given under separate headings.

Part I

The change in fibre volume due to stimulation

A series of four runs is shown in Fig. 5. The fibre swells as the result of stimulation. The amount of the swelling is calculated by reference to the calibration curve recorded for the particular fibre. The results for forty-one runs on eleven different fibres are shown in Table 1. The average change in radius for 10,000 impulses was 0·11 μ. Attention should be drawn to one feature of the series shown in Fig. 5, i.e. the continuation of the swelling for some seconds after the stimulation. However, this is not constant: in some cases it was greater than as shown in Fig. 5, in others there was little or no overshoot. There is also considerable variability in the rate of recovery; on occasions it was nearly complete in 2 min., sometimes the curve overshot the base-line, in other instances the recovery was extremely slow. In view of this lack of consistency it was not thought worthwhile to correct the records by making allowance for the time constant of the circuit. An apparent slow recovery, as in runs I, III and IV of Fig. 5, is largely due to decay of the charge on the coupling condenser, but the recovery in run II is obviously too rapid to be accounted for in the same way. The peaks of the curves are slightly reduced below the true values owing to the decay of the charge.

Apart from those shown in the table, three further runs on one fibre were recorded, giving increments of radius of 0·058, 0·062 and 0·098 μ., but these are not included in the average as they showed an unusual abnormality: the onset of the change was greatly delayed. In one of these runs it did not start until about half-way through the stimulation period, in the other two it had only just commenced at the end. A further four fibres from three *Sepia* failed, for some unknown reason, to show any swelling response; it was certainly less than 0·01 μ., although the possibility of the response being greatly delayed cannot be ruled out, owing to the instability of the base-line. All possible tests were made to confirm that the failure was not instrumental in origin. The only explanation which seems at all likely suggested itself in the last experiment,

during the final stage of the dissection: it was found that the collagenous connective tissue sheath investing the giant fibre itself could in this instance be completely stripped away, although only with considerable difficulty. No attempt had previously been made to remove this sheath. It is possible that its thickness has a considerable influence on the time course of the response;

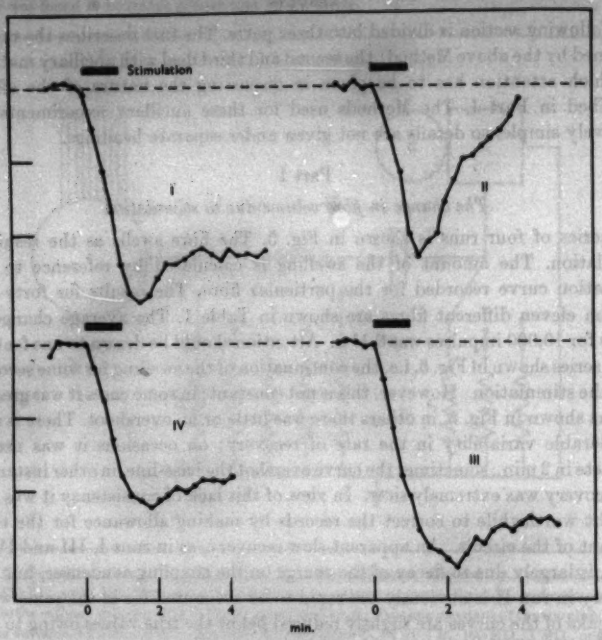

Fig. 5. A series of four runs on the same fibre. The increase of radius (at peak) was as follows: Run I, 0·19 μ.; II, 0·15 μ.; III, 0·18 μ.; IV, 0·14 μ. The nerve was stimulated in each case at 200/sec. for 1 min. (12,000 impulses). Fibre diameter, 238 μ. The time constant of the coupling circuit was 450 sec. The records are not corrected to allow for the time constant.

it may, for instance, determine the time at which the response reaches its peak, and in some cases it might so greatly delay the transference of water (which is probably the cause of the swelling, as will be seen later) as to obscure completely the normal response. It has been found as a general rule in these experiments that an early peak, coincident with the end of the period of stimulation and not delayed, is associated with a change in radius above the average and a relatively rapid recovery. This is what would be expected if the inward diffusion of water and the recovery processes have opposing tendencies. It may be significant

TABLE 1. Change in radius of *Sepia* axon

No. of impulses (thousands)	Change in radius (μ.)	Change in radius per 10,000 impulses (μ.)
6	0·047	0·078
6	0·038	0·063
6	0·040	0·067*
12	0·065	0·054
9	0·052	0·058
12	0·115	0·096
12	0·121	0·101
12	0·048	0·040
12	0·045	0·038
12	0·086	0·072*
12	0·078	0·065
18	0·055	0·031*
12	0·188	0·157
12	0·152	0·127
12	0·182	0·152
12	0·143	0·119
12	0·165	0·138*
12	0·116	0·097*
12	0·117	0·098
12	0·065	0·054
12	0·071	0·059
12	0·103	0·086
12	0·065	0·054
12	0·134	0·112
12	0·061	0·051
12	0·069	0·058
12	0·058	0·048
12	0·058	0·048
6	0·053	0·088*
6	0·057	0·095*
6 †	0·053	0·088*
12	0·091	0·076
12	0·114	0·095
12	0·084	0·070
6	0·156	0·260
6	0·288	0·380
6	0·168	0·280
6 ‡	0·156	0·260
9	0·228	0·254
9	0·216	0·240
9	0·216	0·240
		Mean 0·11 μ.

† Fibre diameter 390 μ. ‡ Fibre diameter 270 μ.

Room temperature in all cases 15–20° C.

Runs for the same fibre are bracketed together. Those above the horizontal line were made in December 1948, the second series, below the line, in March 1949. The swelling and (in the case of the second series) the initial shrinkage also, were observed but not recorded on many other occasions. The mark * indicates low accuracy, due to considerable scatter in the readings. In cases not so marked, the scatter was about the same as in the runs shown in Fig. 5.

Inclusion of a third significant figure in the results for individual runs should not be taken to mean that the 'discrimination' is really 0·001 μ., although it is probably better than 0·01 μ.

that the first stage of recovery in the runs with the fibre stripped of its sheath had an unusually abrupt onset, starting immediately at the end of the period of stimulation, and that this was associated with a change of radius of more than twice the average value. If this interpretation is correct it might account for some of the variability in the results, but it also means that the ionic movements are under-represented in magnitude by the volume change. This point is referred to again later on.

Initial shrinkage

The opacity change in a crustacean nerve trunk (Hill, 1950) indicated that under certain conditions the fibre diameter decreased transitorily before the main swelling phase. In the earlier experiments (December 1948) with the single fibre an initial shrinkage was not observed, but in the later series (March 1949) it was seen on numerous occasions and with great regularity. Three fibres from two *Sepia* showed an early shrinkage in a very striking way, but it was more or less prominent in every fibre of the later batch, including one of those which failed to show the normal swelling response. The initial shrinkage was most marked at the highest rates of stimulation (200/sec.) and could generally not be seen if the rate was reduced below 50/sec. The shrinkage phase lasted only 3–4 sec.; it then reversed into the usual swelling phase, which went through its normal course. The maximum shrinkage only amounted in most instances to about 0·01 μ., but in spite of being so small this change could be easily distinguished against the background disturbance owing to its rapid onset and reversal. It could be repeated many times in quick succession, and there was no doubt at all about its reality. No attempt has been made to record the time course or size of this shrinkage; the present slow recording system is not suitable for the purpose.

Controls

(1) *Stimulation artefact.* A certain proportion of the stimulating current passes through the part of the fibre under observation, and it was necessary to show that the current itself was not responsible for the change. This was done by using a fibre which had become inexcitable, and then passing through the stimulating electrode a current twenty times greater than that normally needed for excitation. No effect could be detected.

(2) *Change in opacity.* The change in light intensity due to stimulation might be caused by an alteration in the opacity or reflecting power of the nerve membrane. This possibility was eliminated by showing that the change in light intensity reversed its sign when the slit edge was shifted from the outer to the inner side of the band of light, to the alternative point of maximum slope on the calibration curve. This result would be consistent only with the edge of the membrane shifting its position, and not with a change in intensity of the scattered light.

(3) *Shortening of the fibre.* It is necessary to consider the possibility that the swelling represents not an increase in volume of the fibre as a whole, but rather a shortening or contraction of the active part of the fibre against the inactive ends of the fibre which lie between the point of attachment to the electrodes and the level of the sea water, the volume of the fibre as a whole remaining constant. To test this, the electrodes were deliberately set at different levels, so that on one side of 18 mm. of active fibre there was 14 mm. of inactive fibre, and on the other side only 3 mm. The fibre was viewed in the centre of the active part, and a small particle which happened to be attached to the membrane was referred to the graduations on a micrometer scale in the eyepiece. A movement of 0·3 μ. could be detected by eye. The fibre was then stimulated for 5 min. at 100/sec. (30,000 impulses). If the active part of the fibre had shortened by 95 μ., which would correspond at constant volume to 0·11 μ. change in radius for 10,000 impulses in a 250 μ. fibre, the reference particle should have been seen to move through about 27 μ. No movement was detectable, i.e. it was less than 0·3 μ. This means that the volume change following stimulation is a real one. In that case, the same observation suggests that the change in length due to the raised internal hydrostatic pressure accompanying the swelling is also very small. As a matter of fact, the length of the fibre probably tends to decrease with stimulation, and the evidence for this is given below. Contact with the glass at the edges of the window prevents this decrease in length from actually taking place.

(4) It has been assumed that the centre of the fibre is fixed, and that the two sides of the fibre move by equal amounts. If this is not so, the average result obtained by the present method, in which the movement of only one side of the fibre is measured, will in the long run be unaffected; but it is possible that some of the variability in the results of Table 1 is accounted for by the movements of the two sides of the fibre being unequal.

Part II

The effect on the length of a nerve fibre of a change in its volume

If the membrane of a nerve fibre were under very small tension, and in its most extensible state, the length of the fibre would be expected to increase when the volume was made to increase; that is to say, Δl is positive for ΔV positive, where l and V represent length and volume. On the other hand, if the membrane is highly stretched, and inextensible, there will be a tendency for Δl to become negative for ΔV positive.

In accordance with this simplified view it is actually found that a giant *Loligo* axon shows either a lengthening or a shortening for ΔV positive, depending upon how much tension there is on the fibre. If the tension is small Δl is positive, if the tension is large Δl is negative. The following experiment illustrates this. A giant *Loligo* fibre of diameter 470 μ., dissected free of small

fibres, was supported in the manner shown in Fig. 6. The platinum rider, suspended on the fibre, could be viewed by means of a microscope with a low-power objective, and a micrometer scale in the eyepiece was arranged to record vertical movements of the rider. Allowance was made for the angle between the two 'limbs' of the nerve, and changes in length of the fibre could thus be measured. The fibre was made to swell by replacing the normal sea water surrounding the nerve by diluted sea water (90% sea water, 10% distilled water), and the change of length recorded. Normal sea water was then returned to the chamber, time allowed for re-equilibration, the rider changed for one of

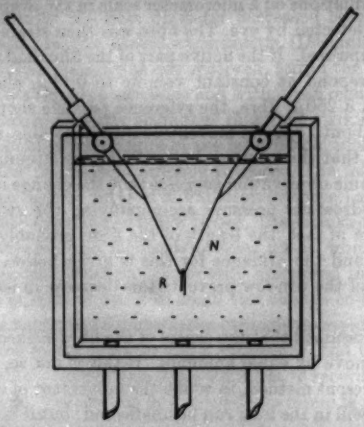

Fig. 6. A single giant nerve fibre, *N*, is held between forceps and weighted at its centre by a platinum rider, *R*. This rider is viewed through the glass side of the vessel by a microscope with a micrometer scale in the eyepiece, and vertical movements of the rider are recorded. The angle between the 'limbs' of the nerve is measured, and the changes in fibre length thus deduced. The solution in the vessel is drained and refilled through the tubes at the bottom.

a different weight, and the process repeated. In order to avoid unduly prolonging the experiment, the change of length, as measured, was not the total change resulting from full equilibration with the new medium, but was taken as the change in length occurring during the interval 0·25–1·25 min. measured from the moment at which the solution was changed. Between runs, 6 min. only was allowed for re-equilibration. Ten runs were taken, with results shown in the table at the head of p. 315. The positive sign denotes an increase in length for ΔV positive, the negative sign a decrease. The results show that at the lowest tension Δl has its greatest positive value, but that it diminishes as the load is increased, reverses when the tension is about 60 mg., and finally becomes negative. (The fibre is not injured by a tension of 300 mg.)

Run	Tension of fibre (mg.)	Change in level of rider (arbitrary units)
1	61	+9
2	309	−27
3	61	+8
4	10·3	+100
5	5·6	+121
6	25	+31
7	309	−39
8	61	+1
9	10·3	+105
10	309	−32

A *Sepia* fibre cannot be made to behave in the same way, for it is not possible, by reducing the tension, to obtain Δl positive for ΔV positive, and even with a tension as low as 5 mg., which is the minimum necessary to keep the fibre under control while the solution is being changed, Δl is negative.

The ratio of $\Delta l : l$ has been measured in a number of instances, and the results are set out in Table 2. The last column gives values for $\Delta V/V$. It is seen later that about 25% of the volume of the fibre is osmotically inactive, and allowance is made for this in calculating $\Delta V/V$.

TABLE 2. Values of $\Delta l/l$ for swelling under reduced osmotic pressure

Date	Diameter of fibre (μ.)	Tension (mg.)	Osmotic pressure of test solution. Normal 100% (%)	$\Delta l/l$ (%)	$\Delta V/V$ (%)
		Loligo			
2. vii. 49	333	Very small	90	+0·33	8·4
4. vii. 49	410	4·9	90	+1·36	8·4
12. vii. 49	350	Zero (no rider)	95	+1·66	4·0
12. vii. 49	350	3·3	95	+0·56	4·0
12. vii. 49	350	17·8	95	Zero	4·0
13. vii. 49	510	2·6	95	+0·93	4·0
13. vii. 49	510	7·2	95	+0·64	4·0
13. vii. 49	510	13·2	95	Zero	4·0
5. viii. 49	515	302	85	−0·63	13·2
11. viii. 49	470	10·3	90	+1·2	8·4
11. viii. 49	470	309	90	−0·43	8·4
		Sepia			
2. viii. 49	180	4·4	85	−2·2	13·2
4. viii. 49	150	4·8	85	−1·2	13·2
8. viii. 49	120	6·2	90	−0·70	8·4
10. viii. 49	150	6·7	90	−0·71	8·4
10. viii. 49	150	6·7	110*	+0·43	−6·8

* Osmotic pressure of sea water raised 10% by addition of 3·28 g. NaCl/l.

When a nerve fibre is made to shrink or swell, the change of length may be much greater than the change of radius (r), and this fact can be used to advantage. For example, with a 150 μ. *Sepia* fibre, $\Delta l/l$ is about −0·8%, for $\Delta V/V$ +8%, and $\Delta r/r$ +4%. A 30 mm. length of fibre therefore shows $\Delta l = 80\ \Delta r$. This 'magnification' is made use of, in the way described below, for measuring the kinetics of water penetration. Measurement of length rather than radius should

also be considered as a possible means of studying the effect of stimulation on the volume of a fibre. A change of 0·1μ. in radius per 10,000 impulses would correspond to 8μ. change of length. But the fibre has to hang free, and the measurement of this change to (say) 10% accuracy (i.e. 0·8μ.) would present certain problems; another difficulty would be that of deducing accurately the value of the volume change from the change of length.

Part III

The kinetics of water penetration following a change in the osmotic pressure of the external solution

There are reasons, which will be discussed later, for supposing that the increase in fibre size following stimulation is due to an intake of water, brought about by a rise in the osmotic pressure of the interior of the fibre. In arriving at this conclusion it is necessary to take account of the rate of penetration of water in response to a change in osmotic pressure, and the kinetics of the process must therefore be determined.

The time course of the volume change following a change in the osmotic pressure was recorded in two ways: (1) by direct measurement of the change of diameter following a sudden replacement of the normal sea water by a solution with an increased or reduced osmotic pressure (this was done both for *Sepia* and *Loligo* fibres); (2) by measuring the change of length following the application of a similar solution.

Method I. Direct measurement of change of diameter

The nerve was mounted, as shown in Fig. 7, and the diameter of the fibre measured by eye on a micrometer scale in the eyepiece of the microscope. The fibre was generally seen to move bodily across the scale while the volume was changing, and it was therefore necessary to record the position of both edges of the fibre when readings were taken. The fibre was slightly stretched between its clamps to reduce movement, and to minimize any change in length. The procedure was to measure the diameter in the 'normal' solution (sea water), then to change the solution by rapidly changing the vessels under the nerve. From that moment readings were made of the diameter at $\frac{1}{4}$ min. intervals for the first 3 min., and subsequently at $\frac{1}{2}$ min. intervals up to 10 min. The original solution was then replaced and a similar series of readings taken as the fibre returned to its normal volume. This cycle was repeated three to five times, and the readings added together.

This method of investigating the kinetics of water penetration was used on three *Loligo* fibres and on one *Sepia* fibre. The curve obtained with the *Sepia* fibre is shown in Fig. 8. The change of length may not have been quite negligible, so the actual magnitude of the change of volume cannot be accurately

determined. However, an attempt was made to estimate the proportion of the volumes of the fibres which may be regarded as osmotically inactive. The figure

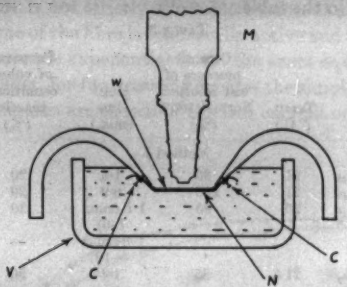

Fig. 7. Apparatus for measuring changes in diameter of giant nerve fibre following changes in the external osmotic pressure. The nerve, *N*, is stretched under the window, *W*, and held with clamps, *C*, *C*. The fibre is viewed by the microscope, *M*, and its diameter measured on a micrometer scale in the eyepiece. The solution around the nerve is changed by removing vessel, *V*, and replacing it by another containing new solution.

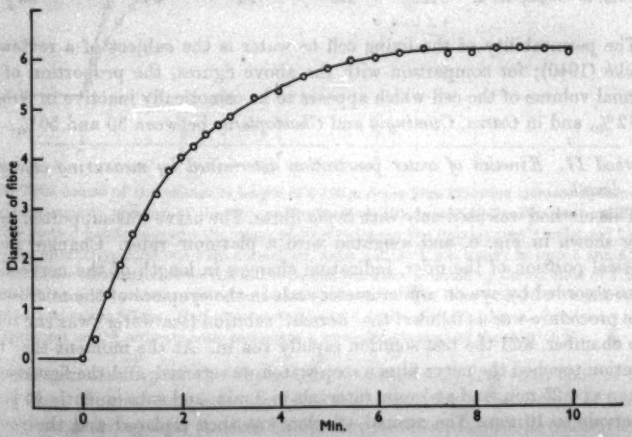

Fig. 8. Time course of the change of fibre diameter following a 15% increase or decrease in external osmotic pressure. Solutions were normal and 85% sea water (30 June 1949). Temperature 21·5° C. *Sepia* fibre, diameter 200 μ. Half-time 1·4 min. It is calculated that 30% of the fibre volume is osmotically inactive. The plotted circles represent the means of observations in six runs. Each unit on the ordinate scale equals 2 μ. change of diameter.

representative of the rate of water penetration is taken as the 'half-time', i.e. the time at which the change of volume is half complete.

The results are shown in Table 3. The kinetics of penetration were also investigated by measuring the change of length (described below) and the results are included in the table.

TABLE 3

Date	Fibre	Temp. (° C.)	Osmotic pressure of test solution. Normal 100% (%)	Half-time (min.)	Proportion of volume osmotically inactive (%)	Half-time, scaled for equal surface volume ratios (arbitrary units)
			Method I			
23. vi. 49	*Loligo*, 290 μ.	21	80	1·4	20	4·8
24. vi. 49	*Loligo*, 320 μ.	21	118	0·9	29	2·8
29. vi. 49	*Loligo*, 400 μ.	26	85	1·1 (runs 1–6)	20	2·8
				0·6 (runs 5–10)	—	1·5
30. vi. 49	*Sepia*, 200 μ.	21·5	85	1·4	30	7·0
			Method II			
					Tension of fibre (mg.)	
4. viii. 49	*Sepia*, 150 μ.	22	85	1·0	4·8	6·7
8. viii. 49	*Sepia*, 120 μ.	20	90	1·1	6·2	9·2
10. viii. 49	*Sepia*, 150 μ.	20	90	1·1	6·7	7·3
10. viii. 49	*Sepia*, 150 μ.	20	110	1·1	6·7	7·3

The permeability of the living cell to water is the subject of a review by Lucké (1940); for comparison with the above figures, the proportion of the normal volume of the cell which appears to be osmotically inactive in *Arbacia* is 12%, and in *Ostrea*, *Cumingia* and *Chaetopterus* between 30 and 50%.

Method II. Kinetics of water penetration determined by measuring change of length

This method was used only with *Sepia* fibres. The nerve was supported in the way shown in Fig. 6, and weighted with a platinum rider. Changes in the vertical position of the rider, indicating changes in length of the nerve fibre, were recorded by eye on a micrometer scale in the eyepiece of the microscope. The procedure was as follows: the 'normal' solution (sea water) was run out of the chamber, and the test solution rapidly run in. At the moment the 'test' solution touched the nerve fibre a stop-watch was started, and the first reading taken at 0·25 min. and at $\frac{1}{4}$ min. intervals to 2 min. and subsequently at $\frac{1}{2}$ min. intervals to 10 min. The normal solution was then replaced and the reverse change recorded. The readings for the two runs (or in some instances, four runs) were added together, to allow for drift, and the results plotted against time. The curve was extrapolated to zero (Fig. 9). It was not possible to determine the position of the rider at zero time by taking a reading before the change of solution, because this change always resulted in considerable disturbance of the nerve and there was a tendency for the rider to move slightly along the nerve. The results are shown in Table 3.

The permeability to water of the membranes of *Loligo* and *Sepia* fibres can be compared by scaling the 'half-times' in proportion to the surface:volume ratios. This procedure is not strictly allowable because it assumes, incorrectly, that the whole volume of the fibre is osmotically active and that the time course of the change has a simple exponential form: the exact equations are given by Lucké (1940). However, for the present purposes the simpler procedure is good enough: the scaled figures are shown in the last column of Table 3. It is seen

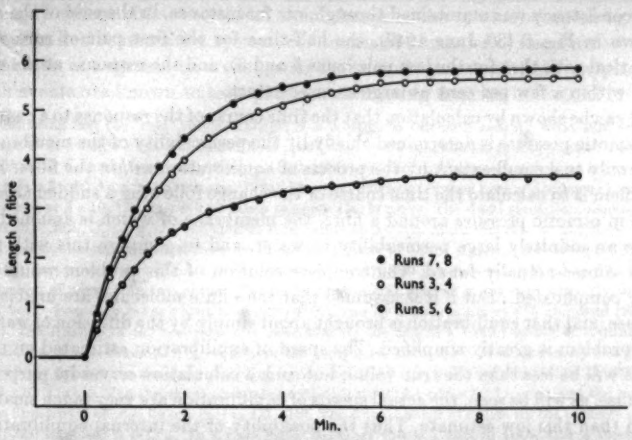

Fig. 9. Time course of the change in length of a 150 μ. *Sepia* fibre following increase or decrease of the osmotic pressure of the surrounding medium (10 August 1949). Temperature 20° C. The plotted points represent the means of observations in two runs: in runs 3 and 4 and 7 and 8 the alternative solutions were normal sea water and 90% sea water; in runs 5 and 6 the alternative solutions were normal sea water and sea water enriched by the addition of 3·28 g. NaCl/l. (osmotic pressure equivalent to 110% sea water). The rider weighed 9·3 mg. and the fibre tension was 6·7 mg. Half-times were: 3 and 4, 1·1 min.; 5 and 6, 1·1 min.; 7 and 8, 1·0 min. Each unit on the ordinate scale represents a vertical movement of the rider of 25 μ.

that the permeability of the *Sepia* fibre is consistently less than that of the *Loligo* fibre. Similar comparison may be made with the permeability to water of other large living cells (Lucké, 1940). For instance, the 100 μ. *Chaetopterus* egg has a half-time of 2 min. and the 76 μ. *Arbacia* egg 6 min. When divided by the surface:volume ratios, the cells being treated as spherical, these figures become (in the arbitrary units of Table 3): *Chaetopterus* 30, *Arbacia* 118. The permeability of these cells is therefore considerably less than the permeability of the giant nerve fibres.

It has been emphasized by Lucké & McCutcheon (1932) that a living cell has to be in perfect condition if it is to show consistent results as an 'osmometer'.

In the experiments just described the excitability of the nerve was not tested, but the reproducibility of the response with any one nerve fibre makes it probable that the fibres were in good condition. The reproducibility does deteriorate after a time, and the response then gets smaller. There is some evidence that before this stage is reached the permeability to water starts to increase. This is shown by the figures for the run on 29 June 1949: the half-time for the combined runs 1–6 was 1·1 min., but for the runs 5–10 had fallen to 0·6 min. The size of the response had started to decline only at the end of the series. On other occasions the consistency was maintained throughout: for instance, in the case of the run shown in Fig. 8 (30 June 1949), the half-time for the first pair of runs was identical with that for the last pair (runs 5 and 6), and the response at the end was within a few per cent as large as at the start.

It can be shown by calculation that the time course of the response to a change in osmotic pressure is determined chiefly by the permeability of the membrane, and only to a small extent by the process of equilibration *within* the fibre. The problem is to calculate the time course of the change following a sudden alteration in osmotic pressure around a fibre, the membrane of which is assumed to have an infinitely large permeability to water, and to compare this with the time course actually found. The complete solution of this problem would be very complicated. But if it is assumed that the solute molecules are unable to diffuse, and that equilibration is brought about simply by the diffusion of water, the problem is greatly simplified. The speed of equilibration estimated on this basis will be less than the true value; but such a calculation serves its purpose, because, as will be seen, the actual speeds of equilibration are very much smaller even than this low estimate. Thus the possibility of the internal equilibration being a factor in determining the overall response may be eliminated. The simplified problem, for a cylinder, is of a standard form (see Hill, 1928). The diffusion coefficient is taken as being that of heavy water into ordinary water, and the value at 20° C. is $2{\cdot}2 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ (Orr & Butler, 1935). The cylinder is assumed to have a diameter of 400 μ. (as for a *Loligo* fibre). The half-time of the equilibration process following a sudden change in the concentration of water at the surface boundary of such a cylinder is calculated to be 1·2 sec. This is very much smaller than the actual half-time of about 60 sec., and it follows that the internal equilibration process is of negligible importance. This conclusion is even more definite in the case of the *Sepia* fibre: the time constant varies inversely as the square of the dimensions, so the half-time of the internal equilibration in a 120 μ. *Sepia* fibre would be only 0·11 sec.

DISCUSSION

It is convenient to draw a distinction between two kinds of change which are associated with activity of a nerve fibre. The action potential itself is associated with a movement across the membrane of inorganic ions (Hodgkin, 1947;

Hodgkin & Huxley, 1947; Hodgkin & Katz, 1949; Keynes, 1950). In addition chemical reactions, or what may be called 'metabolic' events, are known to take place (Gerard, 1932). For instance, prolonged tetanization of nerve leads to a breakdown of combined phosphate (Gerard & Wallen, 1929). Creatine (or arginine) phosphate and adenosine triphosphate are present in nerve, and in many respects the metabolic cycle is similar to that in muscle.

The following calculation makes it seem unlikely that the metabolic reactions are responsible for the observed swelling of a nerve fibre. The volume of the fibre may change either because of a real change in the volume occupied by the molecules in a conservative system, or because of a transfer of water across the membrane in response to a change in the osmotic pressure of the axoplasm. Both events are known to take place in contracting muscle.

Considering first the 'real' volume change, it is possible to calculate roughly what this would amount to in a single fibre. The initial heat production in a 2 sec. tetanus of frog's gastrocnemius muscle at 20° C. can be taken as 0·06 cal./g. (Hill, 1931), and the 'real' decrease in volume associated with this is 3×10^{-5} c.c./g. (Hartmann, 1934). The same relation between heat and volume (1 cal./g. equivalent to 5×10^{-4} c.c./g.) will be taken to apply also in nerve. The squid giant axon respires at about 25 cu.mm. O_2/g./hr. and continuous stimulation at 200/sec. raises this rate by not more than 20% (personal communication from Dr Philip W. Davies, of Johns Hopkins University). Taking 1 c.c. O_2 as equivalent to 5 cal. the heat produced by 720,000 impulses is 0·025 cal./g. The heat production of nerve is in two phases. The initial heat in crab's nerve is not more than 2% of the total heat. Recovery takes about 25 min. (16° C.), and by 1 min. is about 8% complete (Feng, 1936). The fraction of the recovery heat generated by the end of a 1 min. period of continuous stimulation can be taken as 4%, or 6% of the total heat. Thus the heat produced by the giant axon (*Sepia* taken to be the same as *Loligo*) by the end of a 1 min. period of stimulation at 200/sec. is $2{\cdot}5 \times 10^{-5}$ cal./g. The shrinkage associated with this would be $1{\cdot}25 \times 10^{-6}$ c.c./g., and the radius of the fibre would change by one part in $1{\cdot}6 \times 10^{3}$. The actual change of radius (of the opposite sign) for 12,000 impulses is 0·13 μ. In a fibre 250 μ. diameter this is 1 part in 1000, and is therefore incomparably greater than any possible 'real' change in volume of the fibre.

Before discussing the second possible cause of swelling by osmotic pressure differences it is necessary to digress for a moment: it was hinted earlier that the peak value of the swelling may not fully represent any osmotic pressure change produced during activity, owing to the lag in water transference and the supervention of the recovery phase. The kinetics of water penetration have been determined for the resting fibre, and the half-time was found to be 1·1 min. Taking the same figure for the stimulated fibre, and assuming that the osmotic pressure difference builds up at a constant rate during a 1 min. stimulation period, the transference of water can be calculated; it is expressed below as a percentage of the final value at equilibrium:

Time (min.) ...	1·0	1·5	2·0	2·5	3·0	3·5	4·0
Percentage transferred	26	46	60	70	77	83	86

It is plain, from the experimental records, that the peak is delayed, but the delay has been found to be rather variable. However, the form of the response is never like that shown by the above figures, which are calculated on the assumption that there is no recovery: for instance, following a 1 min. period of stimulation the peak is generally found between 1·5 and 2·0 min., whereas the response according to the above calculation is only 50–60% complete in that time. It would seem, therefore, that some form of recovery must be tending to restore the fibre to its original volume. It is suggested that the maximum osmotic pressure difference is three times as great as would correspond to the average change in radius actually recorded. In other words, the equivalent change of radius should be taken as 0·33 μ. per 10,000 impulses.

Considering the second possible cause of swelling by osmotic pressure differences, the heat production may again be taken as a measure of the metabolic activity. It is assumed that the ionic exchanges are thermally neutral. A rough estimate may be made of the number of molecules of metabolite produced by 1 min. of stimulation at 200/sec. It is best to consider the breakdown of arginine phosphate in this connexion, for this reaction is almost certainly one which does take place, and one which is relatively slowly reversed in recovery. Meyerhof (1947) gives 11,000 cal./mole for the heat of breakdown of creatine phosphate (12,000 cal./mole for ATP) or 1 cal. represents the production of $9{\cdot}1 \times 10^{-5}$ mole phosphate. The relation between heat and phosphate for the arginine phosphate of invertebrates may roughly be taken as the same. The heat production up to the end of 1 min. stimulation at 200/sec., $2{\cdot}5 \times 10^{-5}$ cal./g., corresponds to a production of $2{\cdot}3 \times 10^{-9}$ mole phosphate/g. The increment of osmotic pressure due to activity in muscle was found by Hill (1930*a*) to be about twice as great as the value calculated from the amount of phosphate produced. It is as though the original creatine phosphate were osmotically inactive, being bound in some way to other molecules, and the osmotic pressure due to activity therefore rises by twice the expected amount, owing to the formation also of free creatine. The increment of internal osmotic pressure should therefore be taken as equivalent to a production of $4{\cdot}6 \times 10^{-9}$ mole/g. The normal osmotic pressure corresponds to a total ionic concentration of about 1·1 mole/l., or $1{\cdot}1 \times 10^{-3}$ mole/g. Water therefore enters the fibre to the extent of 1 part in $2{\cdot}4 \times 10^{6}$, and the radius changes by 1 part in $4{\cdot}8 \times 10^{8}$. The actual change of radius (of the same sign) is 1 in 300, about 1500 times as large.

Although it would seem unlikely, from the above calculation, that 'metabolic' events can be playing any part in the observed effect, there is one important criticism of the argument. It may not be correct to infer from the low value of the ratio of initial to total heat that the change in osmotic pressure is only 6 % of the maximum possible value (represented by the total heat production) by the end of the 1 min. stimulation period. This assumes that the initial heat in a giant nerve fibre is only 2 % of the total heat, by analogy with crab's nerve; but in medullated nerve the ratio is 10 % and in muscle 50 %. In any case, although there is good reason for supposing that *total* heat is a good index of *total* metabolic turnover, initial heat does not necessarily represent the initial metabolic breakdown. Taking the extreme case, and supposing that the phosphate production is 100 % complete, instead of 6 %, by the end of the stimulation period, the actual increase of radius is greater by a factor of only 90 over the change expected from 'metabolic' reactions. This can hardly be taken as final proof that 'metabolic' events do not play a part, for the calculation has of necessity to be an extremely rough one. An experimental test to distinguish between an increase in osmotic pressure due to metabolic reactions, and that due to ionic exchanges, could be done by finding the effect of reducing to a minimum the volume of external electrolyte. A nerve fibre conducts readily even if it is immersed in paraffin oil, when the remaining external electrolyte layer is only a few microns thick. The change in fibre size under such conditions would be reduced practically to zero if the increase in internal osmotic pressure were due to the production of new particles, as in metabolic breakdown; whereas water transfer caused by ionic exchange across the membrane would be unaffected, and the swelling from this cause would be as large as under normal conditions. Unfortunately this test cannot be made, for if the fibre is immersed in paraffin oil its membrane is no longer visible under dark-field illumination: all the light is reflected from the water-oil interface (the oil having a refractive index greater than that of sea water). It is possible that the test could be made with the fibre in air in a moist chamber, but to do this would involve a complete redesign of the apparatus.

To sum up, it seems reasonable to conclude that metabolic events are unlikely to be the cause of the swelling, even if they cannot be definitely excluded.

The next question to be considered is whether the exchange and transfer of inorganic ions across the membrane are responsible for the swelling of the fibre. The process of excitation probably results in a sudden increase of the permeability to sodium (Hodgkin & Katz, 1949). This results in the rapid discharge of the membrane capacity by the large inward sodium current. The membrane is then

recharged by an outward potassium current and also by an inward current of chloride ions. This means that, in effect, two things take place: (1) internal potassium exchanges with an equal quantity of external sodium; Keynes (1950), using a tracer method, has measured this exchange; it amounts to $4{\cdot}5 \times 10^{-12}$ mole K/sq.cm. membrane surface/impulse; (2) external sodium chloride enters the axon. Keynes (1950) was not able to measure the net exchange of sodium during activity, so the quantity of sodium chloride entering the fibre could not be estimated.

Consider, first, the exchange of sodium and potassium: this should cause the fibre to swell, because the osmotic pressure of sodium is greater than the osmotic pressure of an equivalent quantity of potassium. The importance of this factor has been calculated from tabulated data on the osmotic coefficients of reference solutions (Harned & Owen, 1943), and the difference of the activity coefficients of sodium and potassium chlorides in sea water was measured experimentally, using Hill's (1930*b*) vapour-pressure method. From the tabulated data it can be calculated that an increment of NaCl added to 0·5 molal NaCl is osmotically 5·88% more active than a molecularly equivalent increment of KCl added to 0·5 molal KCl. Experimentally, addition of 100 mM. NaCl to 1 l. of sea water gave a decrease of vapour pressure 3·79% greater than that due to 100 mM. KCl/l. (mean of thirty-two observations). It is convenient to express the difference as the number of water molecules apparently rendered osmotically inactive by the sodium ion (or the excess rendered inactive by sodium over those rendered inactive by potassium). The figure deduced from the tabulated data is 3·1 mol. H_2O per Na ion, and from the vapour pressure measurements it comes to 3·3 mol. H_2O per Na ion. It is not certain whether this number of water molecules is really bound by sodium, or whether the difference in activity is due to some other factor, such as ionic interaction. It is well known that the sodium ion is more heavily hydrated than potassium, and Conway (1947) gives sodium as being hydrated with 8·0 mol. H_2O, and potassium 3·8 mol., a difference of 4·2 (see Bockris (1949) for a critical review of the subject of ionic solvation). For the purpose of this discussion it does not matter whether the expression of activity differences in terms of bound water molecules is real or fictitious, but it is interesting that the water *apparently* bound is so close in amount to that generally assumed to be acting as hydration water.

It may be assumed that the exchange of one sodium with one potassium ion results in the entry of 3·2 mol. of water. It can be calculated that the exchange of $4{\cdot}5 \times 10^{-12}$ mole/cm.^{2}/impulse will, for this reason, result in an increase in fibre radius of $2{\cdot}5 \times 10^{-6}$ μ./impulse. For 10,000 impulses this is 0·03 μ., which is only a small fraction of the 0·33 μ. estimated as representing the total change.

The initial shrinkage which does undoubtedly occur in some circumstances is not easy to explain. One suggestion is as follows. In the rapid exchange of

sodium and potassium, the potassium remains associated with its hydration water, which accompanies the ion through the membrane, and the sodium ion is forced to part with its hydration water, the latter following slowly afterwards under normal osmotic forces to replace that taken up by the sodium when it reaches the other side. If this happens, the first phase should be a shrinkage, and the rate at which it reverses will depend upon the permeability of the membrane to water. The actual shrinkage may well be too large to be accounted for in this way. If excitation involves sodium being entirely stripped of its water, and potassium not at all, the initial decrease in radius, corresponding to an outflow of 3·8 mol. of water per ion, would be only 0·02 μ./10,000 impulses, even if no swelling phase supervened. Actually, the reversal takes place within a few seconds of the start of the stimulation, yet the peak shrinkage is as much as 0·01 μ., even though less than 1000 impulses have passed. On the above hypothesis it is hard to account for a change as large as this.

The above interpretation of the initial shrinkage is not the only possible one, for an alternative explanation, suggested by Hill (1950) to account for an apparent transient shrinkage of the fibres of a crustacean nerve trunk, might also apply here. In the unstimulated fibre, the membrane of which is almost impermeable to sodium ions, dilution must result in a swelling which is permanent, or which regresses only slowly. But if the permeability to sodium is suddenly increased, as by stimulation, sodium and chloride enter the fibre and it swells. But if the interior of the fibre is initially under hydrostatic pressure due to a stretched membrane, there will be a tendency for the fibre to shrink, which could come about in the active fibre by a rapid extrusion of potassium and chloride ions. In this case water would pass out *with* the ions, whereas the water *follows* the ions if they move under concentration gradients. Thus the net ionic exchange may be inwards right from the start, and yet the form of the response be diphasic. This explanation, applied to the initial shrinkage in a *Sepia* fibre, implies that under normal conditions the hydrostatic pressure in this fibre is appreciable. The effect of dilution of the solution on the response to stimulation has not been tested.

There is a change of radius of 0·31 μ. still to be accounted for. Suppose that external sodium chloride enters the axon, and that water also passes into the fibre to maintain the osmotic pressure at the normal value, corresponding to a total ionic concentration of 1100 mM./l. It is calculated that an entry of $1{\cdot}7 \times 10^{-12}$ mole NaCl/cm.^{2}/impulse is sufficient to give an increase in radius of 0·31 μ./10,000 impulses. This ionic *transfer* amounts to 38% of the ionic *exchange*, and the amount of sodium entering the fibre during activity is 38% greater than the amount of potassium leaving, the excess of sodium being accompanied by chloride.

If this figure of 38% is correct, it can be shown that the average permeability of the membrane to potassium during the descending phase of the action

potential is greater than it is at rest, or during the ascending phase. Hodgkin & Katz (1949) have made an estimate of the ionic currents caused by a depolarization of the membrane. At the resting potential these currents are: K, outward, $10{\cdot}6 \times 10^{-11}$ mole/cm.^{2}/sec.; Cl, outward, $2{\cdot}2 \times 10^{-11}$ mole/cm.^{2}/sec. When there is zero potential across the membrane, during the ascending part of the action potential, the currents are: K, outward, $6{\cdot}2 \times 10^{-10}$ mole/cm.^{2}/sec.; Cl, inward, $4{\cdot}2 \times 10^{-10}$ mole/cm.^{2}/sec. The difference due to activity is therefore in the ratio K:Cl = 5·1:4·4. In other words, if the relative permeabilities to potassium and chloride during the recharging phase were the same as at rest or during the ascending phase, the contribution of the chloride current to the recharging of the membrane capacity would be 86% of that due to the potassium, and the entry of sodium 86% greater than the loss of potassium. The actual figure would seem, from the present experiments to be 38%, which would be explained if the permeability to potassium were rather greater —in proportion to the chloride permeability—during the descending phase of the action potential, than it is in the resting state. There is other evidence of an increase in permeability to potassium during the descending phase. Hodgkin & Katz (1949) have shown that the positive after-potential of the squid fibre is more accurately accounted for theoretically if it is assumed that the permeability to potassium, at that stage, is 1·8 times greater than at rest. In a later paper (Hodgkin, Huxley & Katz, 1950) it is further suggested that the potassium permeability is raised above the resting value during the descending phase of the action potential.

Lastly, a few remarks about the nature of recovery from activity, and the volume change which might be expected to accompany it. Recovery, in the complete sense of the word, would involve an active excretion of sodium from the fibre, chloride ions would accompany the sodium and potassium would enter in exchange. The volume change would be completely reversible. If, on the other hand, there is no active excretion, the only possible form of recovery is an inward diffusion of potassium chloride to make up for the deficit of potassium due to activity. This constitutes recovery only to the extent of partially restoring the internal potassium; internal sodium is not decreased, internal chloride is increased still further, and there would be a further swelling of the fibre. As a matter of fact, there are indications (see p. 321) that there is at least partial restoration towards the normal volume, rather than a further increase. A closer study of the time course of recovery might show that in the single fibre the volume change is completely reversible, as it would seem to be in the crustacean nerve trunk (Hill, 1950). In the giant fibre of *Loligo* it seems fairly certain that some provision must exist for the excretion of sodium which leaks in continuously when the fibre is at rest, for Hodgkin & Katz (1949) have produced evidence to show that the permeability of the resting membrane to sodium is not negligible, but amounts to about 4% of the permeability to

potassium; and it seems reasonable to assume that whatever mechanism is operative at rest to excrete sodium will work at a greater rate as the result of activity.

SUMMARY

1. It has been possible to measure an increase in radius of a single cuttlefish nerve fibre as the result of repetitive stimulation. The increase amounts to about 0·1 μ. for 10,000 impulses.

2. The swelling is thought to be due to an increase in the osmotic pressure of the interior of the fibre. This is attributable partly to the exchange of sodium and potassium across the membrane, and partly to the transfer of sodium chloride into the fibre. It is calculated that the sodium *transferred* (with chloride) is about 38% of the amount *exchanged*. Reasons are given which make it unlikely that the increase of osmotic pressure due to 'metabolic' changes within the fibre (e.g. the breakdown of arginine phosphate) is large enough to have a measurable effect.

3. At high rates of stimulation an initial transitory shrinkage may precede the swelling phase. The meaning of this is discussed.

4. The relation between the length and volume of a giant nerve fibre has been investigated. If the fibre is under low tension the length increases when the volume is raised by immersion in a dilute solution; when the tension is higher the length decreases when the volume is increased.

5. A study has been made of the kinetics of the water exchange across the fibre membrane resulting from a change in the external osmotic pressure. The half-time of the process in the single fibre of *Sepia* or *Loligo* is about 1·1 min.

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REFERENCES

Bockris, J. O'M. (1949). *Quart. Rev. Chem. Soc.* **3**, 173.
Cole, K. S. & Curtis, H. J. (1939). *J. gen. Physiol.* **22**, 649.
Conway, E. J. (1947). *Irish. J. Med. Sci.* (Oct.–Nov.).
Feng, T. P. (1936). *Ergebn. Physiol.* **38**, 73.
Gerard, R. W. (1932). *Physiol. Rev.* **12**, 469.
Gerard, R. W. & Wallen, J. (1929). *Amer. J. Physiol.* **89**, 108.
Harned, H. S. & Owen, B. B. (1943). *The Physical Chemistry of Electrolytic Solutions.* Reinhold Publishing Corporation.
Hartmann, H. (1934). *Biochem. Z.* **270**, 172.
Hill, A. V. (1928). *Proc. Roy. Soc.* B, **104**, 71.
Hill, A. V. (1930*a*). *Proc. Roy. Soc.* B, **106**, 445.
Hill, A. V. (1930*b*). *Proc. Roy. Soc.* A, **127**, 9.
Hill, A. V. (1931). *Proc. Roy. Soc.* B, **109**, 267.
Hill, D. K. (1948). *J. Physiol.* **107**, 479.
Hill, D. K. (1950). *J. Physiol.* **111**, 283.
Hill, D. K. & Keynes, R. D. (1949). *J. Physiol.* **108**, 278.
Hodgkin, A. L. (1947). *J. Physiol.* **106**, 319.

Hodgkin, A. L. & Huxley, A. F. (1947). *J. Physiol.* **106**, 341.
Hodgkin, A. L., Huxley, A. F. & Katz, B. (1950). (To be published.)
Hodgkin, A. L. & Katz, B. (1949). *J. Physiol.* **108**, 37.
Keynes, R. D. (1950). *J. Physiol.* (in the Press).
Lucké, B. (1940). *Symp. quant. Biol.* **8**, 123.
Lucké, B. & McCutcheon, M. (1932). *Physiol. Rev.* **12**, 68.
Meyerhof, O. (1947). *Ann. N.Y. Acad. Sci.* **47**, 815.
Orr, W. J. C. & Butler, J. A. V. (1935). *J. Chem. Soc.* p. 1273.
Young, J. Z. (1936). *Proc. Roy. Soc.* B, **121**, 319.

J. Physiol. (1950) 111, 328–334 612.492:612.112

PITUITARY GLAND AND BLOOD LYMPHOCYTES

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It has been shown that injection of adrenocorticotrophic hormone (A.C.T.H.) in mice, rats and rabbits produces a temporary fall in the absolute number of blood lymphocytes (Dougherty & White, 1943, 1944). This response would appear to be mediated by the adrenal cortex, for injection of extracts of the adrenal cortex produce a similar lymphopenia, whereas injection of A.C.T.H. into adrenalectomized animals has no effect (Dougherty & White, 1944). These results have been confirmed by other workers. For details and discussion see recent reviews by Valentine, Craddock & Lawrence (1948), and White (1949).

It thus seemed possible that the production of a temporary lymphopenia might be used as an indicator of the secretion of A.C.T.H. by the anterior pituitary gland. In preliminary experiments electrical stimulation of various regions of the hypothalamus and pituitary gland was performed by the remote control method in unanaesthetized rabbits to see whether a lymphopenia might be so produced. For this method of stimulation (Harris, 1948*a*) it was necessary to immobilize the rabbits in clamps for periods up to 2 hr. These experiments were stopped, however, when it was found that the emotional stress involved by immobilizing the animals in clamps (without electrical stimulation) was sufficient to produce the lymphopenic response. This paper deals with the part played by the pituitary gland in this reaction to emotional stress. The effects of electrical stimulation of the hypothalamus and hypophysis by a technique that does not require immobilization of the animal form a further report.

METHODS

Blood counts. Adult rabbits of both sexes, body weight 2–3 kg., were used. The total white cell count was made using the Thoma form of haemocytometer, diluting the blood ten times with 1% acetic acid coloured with gentian violet, and counting the number of cells in 0·02 cu.mm. of diluent. The differential count was made by counting a total of at least 200 cells (stained with Leishman's stain) under a $\frac{1}{12}$ in. oil immersion objective. It was found convenient to use an electric counting device, foot operated, in recording the number of cells. The figures obtained in this way from counts on forty-five normal rabbits are: total white blood corpuscles per cu.mm. 10,238 ± 244 (S.E. of mean of sixty-three observations) (Dougherty & White, 1944—10,053 ± 1730/cu.mm.), and absolute lymphocytes per cu.mm. 6841 ± 161 (63) (Dougherty & White, 1944—6861 ± 1723/cu.mm.).

Emotional stress. In the preliminary experiments the animals were placed in restraining clamps, as described by Harris (1948*a*), for periods of 1 or 2 hr. In all later experiments the emotional stress stimulus was administered by passing a faradic current, from an inductorium, through two needle electrodes inserted subcutaneously over the lumbar vertebrae (O'Connor & Verney, 1942). In most experiments this stimulus was administered twice, for periods of 5 min. (1 min. on, 1 min. off), with half an hour interval. The strength of the stimulus used was that which just produced definite signs of fright on the part of the animal, such as crying, struggling, tachycardia, and polypnoea. The full procedure was as follows. The rabbit, which had been left undisturbed overnight, was removed from its cage and the initial blood sample taken from the marginal vein of the ear. Two needle electrodes were then inserted under the skin and the faradic stimulus administered. Further blood samples were taken hourly for 3 hr. and usually again at the sixth hour, the animal being replaced in its cage in the intervals.

Operative procedures. Hypophysectomy was performed by the parapharyngeal method of Jacobsohn & Westman (1940), which was found to be very satisfactory. The first few days after operation the animals were often disinclined to take food, and during this period many died from hypoglycaemia on the second or third day, in spite of glucose administration. The mortality of the animals that survived the first few post-operative days, and started eating again, was very low. Denervation of the adrenal glands was performed through a peritoneal approach and involved removal of the splanchnic nerves and upper part of the lumbar sympathetic chain on both sides.

Histology. The routine procedure consisted of killing the animal with chloroform, injecting the carotid arteries with 150 c.c. of a one in three dilution of indian ink, fixing the head in formalin, decalcifying in Bensley's solution, and embedding a block of tissue containing the base of the skull, sella turcica and hypothalamus in celloidin. Serial sections, 100–150 μ. thick, were cut and stained with haemalum and eosin.

RESULTS

Emotional stress stimulus in normal rabbits

In seven preliminary experiments on five rabbits the animals were placed in immobilizing clamps for periods of 2 hr. It was found that this immobilization produced a lymphopenia in all cases.

In order to standardize the emotional stimulus the method of O'Connor & Verney (1942) was adopted in most of the later experiments. It was found that the maximum drop in blood lymphocytes occurred at about the third hour after the emotional stress stimulus. In these and the later experiments the results have been expressed by giving the number of lymphocytes per cu.mm. at the third hour as a percentage of the initial count. Thus a result of 100% indicates no change in the lymphocyte count at the third hour after the emotional stress stimulus, whilst a result of 50% indicates a drop in blood lymphocytes to one-half the initial number. It has been assumed that in this way figures are obtained which are comparable between animals showing a difference in their initial (resting) lymphocyte counts.

In all of forty-four experiments on forty-two normal rabbits a temporary lymphopenia was observed to follow emotional stress. The lymphocyte counts at the third hour were 69·2% ± 1·8 (44) of the initial counts. After a further 3 hr. the lymphocyte count was returning, or had returned, to the initial level. Of these forty-two normal rabbits, thirty-one were female and eleven were male. No sex difference in the lymphopenic response to emotional stress was

found, the average results in the female rabbits being 68·4% ± 1·9 (32), and in the male rabbits 71·3% ± 2·2 (12). Two experiments on adult rabbits that had been gonadectomized when a few weeks old, gave results of 53% (castrate male) and 64% (spayed female). These figures suggest that the gonads are not directly involved in the reaction.

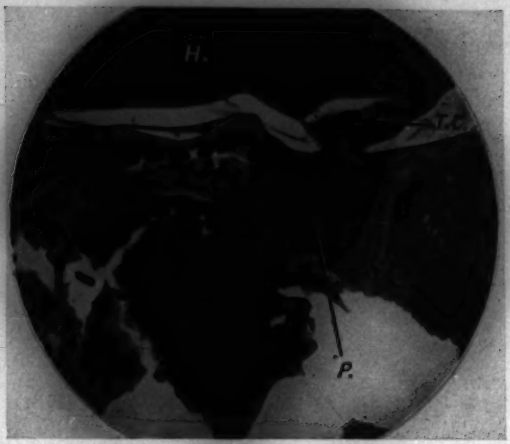
A study of the other blood cells, following an emotional stress stimulus, showed that a granulocytosis occurred in the majority of cases, and that the erythrocyte count remained unaltered. This latter observation may be taken as evidence that the lymphopenia was not due to changes in blood volume.

Emotional stress stimulus in hypophysectomized rabbits

The operation of hypophysectomy is followed by a fall in the resting level of blood lymphocytes. In nine rabbits the preoperative lymphocyte count per cu.mm. was 6917 ± 333 (18), and on the first post-operative day 4611 ± 496 (9). In those animals which survived, the pre-operative level of lymphocytes was regained within 1–4 weeks. A similar reduction in the blood lymphocytes has been observed to follow exposure of the pituitary gland without removal (in blank operations), and also following operations to denervate the adrenal glands. Therefore this reaction appears associated with trauma rather than a specific loss of the pituitary gland, and could be interpreted as being part of the picture of the alarm reaction described by Selye (1946).

Fourteen rabbits were submitted to emotional stress stimuli following the operation for hypophysectomy. During the first two post-operative weeks, twenty-six experiments were performed on twelve of these rabbits, and in no case was a definite lymphopenic response observed. Previous to operation ten of these animals had been tested, and responded normally. After the second post-operative week seventeen experiments were performed on eight rabbits, and it was found that four of these animals showed a continued lack of response, whilst the other four animals showed a gradual return of the response to emotional stress. Microscopic examination of serial sections through the sella turcica revealed the hypophysectomy to have been incomplete in the four cases that showed a return of the response. It was of interest that the pituitary fragments left *in situ* in these animals still retained, or had reacquired, vascular connexion with the tuber cinereum through the hypophysial portal vessels (Pl. 1).

At first it seemed possible that the loss of lymphopenic response in the early post-operative days was associated with the low resting level of blood lymphocytes. This is unlikely as (*a*) injection of anterior pituitary extract or adrenocorticotrophic hormone still elicited the usual lymphopenic response in these animals (see below), and (*b*) two rabbits in which the pituitary gland had been exposed but not damaged, and which showed a similar fall in the resting lymphocyte level after operation, still responded with the usual acute decrease



Microphotograph of a midline sagittal section through hypothalamus and base of skull (including sella turcica) of an incompletely hypophysectomized rabbit (180). The vascular system of this rabbit was perfused with indian ink immediately after death. Note that the residual fragment of anterior pituitary tissue (*P.*) is connected to the tuber cinereum (*T.C.*) of the hypothalamus (*H.*) by blood vessels. The outline of the sella turcica is indicated by the dotted line. Magnification, × 12.

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after emotional stress. It is probable that the negative responses of the incompletely hypophysectomized rabbits in the first two post-operative weeks were due to lack of vascular connexions to the retained fragments of the gland.

TABLE 1. To show the effect of emotional stress on blood lymphocytes following the operation for hypophysectomy. The figures given express the blood lymphocytes at the third hour following the emotional stimulus as a percentage of the initial count. Note that in the completely hypophysectomized animals no definite lymphopenic response follows emotional stress, and that in some cases even a lymphocytosis occurs

	Lymphopenic response					
		Post-operative				
Rabbit	Pre-operative	1st week	2nd week	3rd week	4th week and later	Operative result
174	—	107, 102, 100	100	—	—	C
176	80	106, 103, 99	102, 103	53	81	I
177	—	—	—	101	57	I
179	57	97	—	—	—	C
180	31	99, 107	97	—	87	I
181	58	102	—	—	—	C
192, castrate male	53	123	—	—	—	C
194	66	112, 102	101	101	—	C
197	74	100	—	—	—	C
200	76	127, 111	—	102	98	C
202, spayed female	64	107, 144	—	—	—	C
203	58	103	119	83	—	I
215	—	—	—	102	118, 115, 125, 112	C
221	—	—	108	114	106, 108	C

I, incompletely hypophysectomized; C, completely hypophysectomized.

To see whether the more peripheral mechanism underlying the lymphopenic response to stress was still capable of reacting after hypophysectomy, the response of operated animals to intravenous injections of anterior pituitary extracts was studied. The results of these experiments are given in Table 2,

TABLE 2. The lymphopenic response following injection of crude anterior pituitary extract or purified adrenocorticotrophic hormone

Rabbit	I.V. injection	Lymphocyte count 3 hr. after injection expressed as percentage of initial count
180, I	3 mg. crude ant. pit. ext.	70
203, I	3 mg. crude ant. pit. ext.	38
194, C	500 μg. A.C.T.H.	73
197, C	250 μg. A.C.T.H.	70
200, C	500 μg. A.C.T.H.	68
202, C, S	500 μg. A.C.T.H.	58

I, incompletely hypophysectomized; C, completely hypophysectomized; S, spayed.

and it may be seen that two incompletely hypophysectomized, and three completely hypophysectomized rabbits gave definite lymphopenic responses

after injection of crude anterior pituitary extract and purified A.C.T.H., respectively. These observations were made during the first two post-operative weeks when all animals failed to show a response to the emotional stress stimulus.

The granulocytosis, which was seen to accompany the lymphopenia after stress in the majority of normal rabbits, still occurred in the hypophysectomized animals.

The relationship of the adrenal medulla

Since the adrenal medulla is excited by emotion, and adrenaline is thought to exert a stimulating effect on the anterior pituitary gland and on the adrenal cortex (Vogt, 1944, 1945; Long, 1947), it was decided to investigate the effect of intravenous injection of adrenaline on the blood lymphocytes, and the effect of denervation of the adrenal glands on the lymphopenic response following emotional stress.

Injection of adrenaline. Intravenous administration of 1 c.c. of 1/150,000 solution of adrenaline hydrochloride in seven normal rabbits resulted in a definite lymphopenic response in one case (66%), one doubtful response (87%) and five negative responses (107, 99, 101, 98, 109%). Control injections of 0·9% sodium chloride made intravenously into six rabbits failed to produce a lymphopenic response (97, 102, 102, 101, 97, 96%).

Emotional stress response after denervation of the adrenal glands. In four rabbits the adrenal glands were denervated. In six experiments on these animals the lymphopenic response (66, 60, 85, 72, 62, 70%) to the usual emotional stress stimulus did not differ significantly from the response in normal animals.

DISCUSSION

It is well known that emotional stress produces a lymphopenia in mice and rats (Harlow & Selye, 1937; Elmadjian & Pincus, 1945; Latta & Nelson, 1948) and man (Pincus, 1947; Mikkelsen & Hutchens, 1948). Little work appears to have been done on this reaction in the rabbit. Cheng (1930) and Nice & Katz (1936) report that restraint in the abdomen-up position or a subcutaneous faradic stimulus in the rabbit results in a leucopenia which according to Cheng is a lymphopenia. In the present work it has beeen found that restraint or subcutaneous faradism in rabbits results in a lymphopenia which is generally associated with a granulocytosis.

The assumption is usually made that stress lymphopenia is due to activation of the anterior pituitary : adrenal cortex mechanism. No detailed study has been made of the part played by the pituitary gland in this reaction. As a first step in such a study it was thought important to see whether hypophysectomy abolished the response. There can be no doubt that such is the case in the rabbit, and the control experiments in which pituitary extract was injected into hypophysectomized animals show that the peripheral mechanism is still

capable of being stimulated, and producing a lymphopenia, for at least 10 days after hypophysectomy. These results are in general agreement with those of Sayers & Sayers (1948), who studied the relationship of stress to the content of sudanophilic substance and ascorbic acid in the adrenal cortex of the rat.

It seems likely that emotional stress excites the adenohypophysis through the mediation of the hypophysial portal vessels (Harris, 1948*b*) and it is significant, in this respect, that the incompletely hypophysectomized rabbits were found to possess vascular connexions between the tuber cinereum and retained pituitary tissue (Pl. 1). The possibility that emotional stress stimulates the anterior pituitary gland via the splanchnic nerves, adrenal medulla and adrenaline secretion, according to the general hypothesis of Long (1947), is thought to be unlikely since a normal stress lymphopenia occurred after adrenal denervation and since intravenous injection of adrenaline did not usually elicit a lymphopenia.

The lymphopenic response is the quickest indicator of possible anterior pituitary stimulation available at present. For experimental work this reaction has the obvious advantages of (i) short time relations, (ii) easy and quick measurement, (iii) absence of anaesthesia and operative interference, and (iv) easy repetition of experiment in the same animal. Care must be taken in using lymphopenia as an indicator of pituitary activity that the animal suffers no incidental emotional stress throughout the experiment and has been quiet and at rest for the previous 12 hr. Provided that precautions are observed it is thought that the lymphopenic response will be of great value in elucidating the factors governing anterior pituitary secretion.

SUMMARY

1. Emotional stress (restraint or subcutaneous faradic shocks) in normal rabbits resulted in a marked absolute lymphopenia which was generally accompanied by an absolute granulocytosis. The lymphopenia was maximum at about the third hour after the stimulus.

2. Emotional stress in hypophysectomized rabbits failed to produce any lymphopenia, though the granulocytosis was not abolished. Intravenous injection of pituitary extract still evoked a lymphopenia after hypophysectomy.

3. Denervation of the adrenal glands did not alter the lymphopenic response to stress. Intravenous injection of adrenaline did not produce a lymphopenia in the majority of animals. It is concluded that the adrenal medulla plays little part in the reaction.

4. The lymphopenic response is the quickest indicator of anterior pituitary activity known at present.

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REFERENCES

Cheng, S. C. (1930). *Amer. J. Hyg.* **11**, 449.
Dougherty, T. F. & White, A. (1943). *Science*, **98**, 387.
Dougherty, T. F. & White, A. (1944). *Endocrinology*, **35**, 1.
Elmadjian, F. & Pincus, G. (1945). *Endocrinology*, **37**, 47.
Harlow, C. M. & Selye, H. (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 141.
Harris, G. W. (1948*a*). *J. Physiol.* **107**, 418.
Harris, G. W. (1948*b*). *Physiol. Rev.* **28**, 139.
Jacobsohn, D. & Westman, A. (1940). *Acta Physiol. Scand.* **1**, 71.
Latta, J. S. & Nelson, W. W. (1948). *Amer. J. Anat.* **82**, 321.
Long, C. N. H. (1947). *Fed. Proc.* **6**, 461.
Mikkelsen, W. P. & Hutchens, T. T. (1948). *Endocrinology*, **42**, 394.
Nice, L. B. & Katz, H. L. (1936). *Amer. J. Physiol.* **117**, 571.
O'Connor, W. J. & Verney, E. B. (1942). *Quart. J. exp. Physiol.* **31**, 393.
Pincus, G. (1947). *Rec. Progr. Horm. Res.* **1**, 123.
Sayers, G. & Sayers, M. A. (1948). *Rec. Progr. Horm. Res.* **2**, 81.
Selye, H. (1946). *J. clin. Endocrinol.* **6**, 117.
Valentine, W. M., Craddock, C. G. & Lawrence, J. S. (1948). *Blood*, **3**, 729.
Vogt, M. (1944). *J. Physiol.* **103**, 317.
Vogt, M. (1945). *J. Physiol.* **104**, 60.
White, A. (1949). *Ann. Rev. Physiol.* **11**, 355.

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HYPOTHALAMIC CONTROL OF THE ANTERIOR PITUITARY GLAND AND BLOOD LYMPHOCYTES

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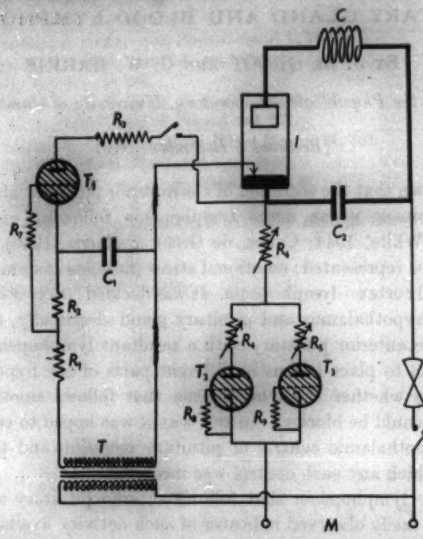
It has been shown that the secretion of the anterior pituitary gland is essential to the development of an acute lymphopenia following emotional stress (Dougherty & White, 1944; Colfer, de Groot & Harris, 1950). The probable sequence may be represented: emotional stress (nervous system)—adenohypophysis—adrenal cortex—lymphopenia. It was decided: (*a*) to stimulate various regions of the hypothalamus and pituitary gland electrically, to see whether secretion by the anterior pituitary with a resultant lymphopenia could be so induced; and (*b*) to place lesions in different parts of the hypothalamus and pituitary to see whether the lymphopenia that follows emotional stress in normal rabbits could be blocked. In this way it was hoped to obtain evidence relevant to hypothalamic control of pituitary secretion and the anatomical pathways by which any such control was mediated.

Although the lymphopenia that follows anterior pituitary secretion is the most rapid and easily observed indicator of such activity available, care must be taken in using this reaction that the animal is not subjected to incidental emotional stress in the course of the experiment. Therefore, in order to stimulate the hypothalamus and pituitary gland electrically in the unanaesthetized *quiescent* rabbit, it became necessary to use a specialized technique.

METHODS

Adult rabbits, between 2 and 3 kg. body weight, and usually female, were used. The method of performing blood counts and inducing emotional stress was as described by Colfer, de Groot & Harris (1950). Electrical stimulation of various regions in the hypothalamus and pituitary gland was performed by the remote control method in the conscious unrestrained animal. The implanted units were similar to those described previously (Harris, 1947*b*). In order to stimulate the animal whilst free to move in its usual cage, a large primary coil 3 ft. in diameter was used. A primary coil of these dimensions entails the use of high amperage current pulses, and to obtain these a modification of the method of Chaffee & Light (1934) was adopted. A single primary coil in the horizontal plane was used, since rabbits do not learn to rotate their heads voluntarily out of the plane of the coil. Short-lived variations in intensity of stimulation (due to movements of the head in eating or grooming the fur) were thought to be compensated by the prolonged periods of stimulation used

in these experiments, that is periods of 1 hr. The current pulses in the primary coil were produced by a circuit (Text-fig. 1) in which a 70 μF. condenser bank (C_2), charged from the mains (360 V. r.m.s.; 50 cyc./sec.) through two thyratrons (T_2, T_3, arranged in parallel and acting as rectifiers) to 500 V. is discharged at a later stage in the cycle through the ignitron (I) and the primary coil (C). The peak value of the current pulses through the primary coil is about 600 amp., and they recur at the frequency of the supply mains, 50 cyc./sec., i.e. at intervals of 20 m.sec. The point of discharge of condenser (C_2) is arranged to be about 15 msec. after the start of the charging pulse, and depends



Text-fig. 1. The primary circuit. The main charging-discharging circuit is on the right in the diagram, and the trigger circuit on the left. C, primary coil of $\frac{1}{2}$ in. copper tubing, 5 turns 3 ft. diameter. All connexions in heavy lines are made of $\frac{1}{2}$ in. copper tubing or 1 in. copper ribbon. C_1, 1 μF. condenser and C_2, 70 μF. condenser bank (condensers suitable for operation in a.c.); F, fuse; I, ignitron BK 24, water-cooled; M, mains supply, 360 V. r.m.s., 50 cyc./sec.; R_1, 1000 Ω.; R_2, 200 Ω.; R_3, 40 Ω.; R_4, R_5, R_6, 0–40 Ω.; R_7, R_8, R_9, 10 kΩ.; T, 1:1$\frac{1}{2}$ step-up mains transformer; T_1, T_2, T_3, thyratron valves, BT 5.

on a high voltage pulse (760 V.) being delivered through the ignitor or trigger circuit. This trigger pulse is obtained by charging condenser (C_1) via a 1:1$\frac{1}{2}$ step-up transformer from the mains, and then discharging through the thyratron (T_1) and ignitor of the ignitron, the timing of the discharge being determined by the phase difference in potential applied to the cathode and grid of the thyratron (T_1). Important technical points are that the connexions shown in heavy type in Text-fig. 1 have sufficient current-carrying capacity, that the ignitron be water-cooled, and that the condensers (C_1 and C_2) and resistances (R_4, R_5 and R_6) are able to withstand the applied voltages and currents. The mean current in the trigger circuit is about 0·14 amp., and in the ignitron circuit 2–3 amp. A small secondary coil similar to those implanted in the rabbits was found to develop a peak potential of 7·0 V. when placed in the centre of the field of the primary coil, and of 13·5 V.

when placed at the periphery of the field almost in contact with the primary coil. By restricting the size of the wooden cage containing the animal in the field of the primary coil, the induced voltage varies less than 2% for different positions in the cage. If a metal cage (of galvanized iron) is used, variations of up to 20% in the induced voltage may occur. Pl. 1, fig. 1, shows the waveform of the pulse induced in a secondary coil. Stimulation of the hypothalamo-hypophysial region by this method produces the same visible signs in the rabbit as described previously (Harris, 1947*b*). The effect on the orbital structures is of value in standardizing the strength of the stimulus between different animals. The strength of stimulation may be most easily adjusted either by varying the resistance R_2 and thus the potential to which condenser C_2 is charged, or by varying the position of the animal's cage in the vertical plane of the primary coil. Electrical stimuli of this type have been shown previously to have a spread of ½ mm. or less from the electrode tip so far as unmyelinated fibres of the supraoptico-hypophysial tract are concerned (Harris, 1947*b*, 1948*a*), and it seems likely that the effective spread for other unmyelinated hypothalamic fibres is of the same order of magnitude. The routine procedure was to place the animal in its cage within the primary coil and to find the appropriate strength of stimulus the evening before the experiment. The following day the initial blood sample was taken, stimulation applied for 1 hr., and further blood samples removed at 1½ hr. intervals from starting stimulation. On the day of the experiment the animals were starved until the last blood sample had been taken. At least three experiments were performed on each animal, and usually on three consecutive days without removing the animal from within the primary coil, so that it became accustomed to the procedure and to its surroundings.

Electrolytic lesions have been placed in various parts of the hypophysis and hypothalamus. In preliminary experiments, the hypophysis was exposed from below by the parapharyngeal route of Jacobsohn & Westman (1940), a platinum electrode (anode) inserted into the gland and a direct current passed for 1 min. In all the later experiments that we describe below, the electrolytic lesion was made with the use of a stereotaxic instrument (Harris, 1937), the electrode insulated to within 0·5 mm. of its tip being inserted through a small trephine hole at bregma. The indifferent cathode was inserted rectally, and a direct current of 1·0 or 3·0 mA. passed for 1 min. This latter method was finally chosen as it involved less operative trauma, less risk of damage to the venous drainage of the gland and gave greater accuracy in placing the hypothalamic lesions. The lymphopenic response to the emotional stress stimulus was measured before operation and again on the first and third post-operative days, the animal being killed at the end of the last experiment.

Other operations. Cervical sympathectomy entailed bilateral removal of the superior cervical ganglion and upper part of the cervical sympathetic chain. In many excitable animals the caudal margin of each ear was denervated by removal of the great auricular nerve in order to facilitate the removal of blood.

Histological procedures. The rabbits bearing implanted coils were killed with chloroform, injected with 5% formol through the abdominal aorta, the head removed, trimmed and placed in 5% formol. The rabbits with electrolytic lesions were killed with chloroform, injected with 150 c.c. 1:3 indian ink solution through the carotid arteries, the head removed, trimmed and placed in 5% formol. After complete fixation all heads were decalcified in Bensley's solution, and a block of tissue, containing hypothalamus, base of skull and pituitary gland, embedded in celloidin. Serial sections, 100–250 μ. were cut and stained with haemalum and eosin.

RESULTS

Stimulation of hypothalamus and pituitary gland

Eighty-three experiments have been performed on fourteen rabbits in which different parts of the hypothalamus and pituitary gland have been stimulated electrically for hourly periods and the effect on the blood lymphocytes studied (see Table 1). The effect of stimulation on the lymphocyte count is expressed by giving the lymphocyte count at the third hour after beginning the stimulus

as a percentage of the initial (pre-stimulus) count. From the results of these experiments the animals fall into two groups.

TABLE 1. Lymphopenic responses to electrical stimulation of various regions of the hypothalamus and pituitary gland. The lymphopenic response is expressed by giving the lymphocyte count at the third hour after starting stimulation as a percentage of the initial count

Rabbit	Lymphopenic responses to electrical stimulation	Average	Response to emotional stress stimulus	Electrode position
152	80, 101, 113, 96, 113, 112	103	41	Between zona tuberalis and main part of pars distalis
161	66, 63, 78, 70, 82, 75, 75, 82, 64	73	78	Posterior region of tuber cinereum, slightly to right of midline
208	76, 94, 84, 85, 100, 105, 90, 109, 98, 92	93	77	In contact with right side of infundibular stem and into pars distalis and intermedia
210	111, 98, 85, 93, 103, 87, 86, 90	94	—	Through supraopticohypophysial tract in median eminence and in pars tuberalis*
214	98, 79, 89, 97, 100, 108	95	75	Pars distalis
219	67, 75, 73, 80, 82	75	52	Posterior region of tuber cinereum midline
222	92, 88, 89, 116, 106, 99, 91	97	73	Lateral tuber cinereum and in zona tuberalis
232	86, 90, 107, 114, 95, 114	101	77	High in tuber cinereum, 1·5 mm. above infundibular stem
237	98, 94, 93, 84, 99, 82, 73, 97, 112	92	62	In supraopticohypophysial tract in median eminence*
258	107, 98, 102	102	68	Third ventricle 0·5 mm. above infundibular stem
271	93, 96, 104	98	68	Left wall of tuber cinereum
272	54, 66, 65, 49, c.s., 66†, 63†	59	55	Mammillary body and in junction of infundibular stem and infundibular process
273	78, 80, 70, 62, c.s., 54†, 68†	73	68	Right posterior region of tuber cinereum*
274	96, 95, 93	95	66	Pars distalis and intermedia and adjacent to right side of infundibular stem*

c.s., cervical sympathectomy.

* See Pl. 1, figs. 2, 5.

† These figures excluded when calculating average of responses.

Positive response. In twenty-two experiments on four animals (161, 219, 272, 273) a marked fall in blood lymphocytes was observed to follow stimulation. This lymphopenic response was very similar in time relations and magnitude to that following the application of an emotional stress stimulus in these and other animals. The onset was acute, the maximal fall in number of lymphocytes occurred at about the third hour, and by the sixth hour the initial level was returning or had been regained. Microscopic examination of the brain and pituitary gland showed the electrode tip to have been situated in the posterior

region of the tuber cinereum (161, 219, 273) (Pl. 1, fig. 3) and in the mammillary body and neurohypophysis (272). The responses to stimulation in these rabbits were quite constant; a definite lymphopenia followed each period of stimulation.

Negative response. Sixty-one experiments on the other ten rabbits failed to demonstrate consistent lymphopenic responses in any animal. An occasional lymphopenia was observed in several of these rabbits, but in none was it constantly obtained. The following regions of the hypothalamus and pituitary gland were negative to stimulation: high in tuber cinereum, 1·5 mm. above the infundibular stem (232); anterior part of the tuber cinereum including the median eminence and the supraopticohypophysial tract (210, 237) (Pl. 1, figs. 2, 4); the lateral region of the tuber cinereum (222, 271); the infundibular stem (208, 274) (Pl. 1, fig. 5); the pars tuberalis and zona tuberalis (152, 210, 222); the pars intermedia (208, 274); and the pars distalis of the pituitary gland (152, 208, 214, 274).

Two rabbits (272, 273), that gave a marked lymphopenia following stimulation of the posterior region of the tuber cinereum, were submitted to bilateral cervical sympathectomy. On the first and fourth days after sympathectomy both animals were again stimulated for 1 hr., and the lymphopenic response was found to be unaltered (Table 1).

Lesions in hypothalamus and pituitary gland

Lesions have been made in various parts of the pituitary gland and hypothalamus in twenty-five rabbits and the effect of an emotional stress stimulus on the blood lymphocytes studied. The effect of an emotional stress stimulus on the lymphocyte count is expressed by giving the lymphocyte count at the third hour after beginning the stimulus as a percentage of the initial count. Before operation the lymphopenic response of twenty-three of these animals to the usual emotional stress stimulus was $69{\cdot}1 \pm 2{\cdot}3$ (S.E. of mean of twenty-three observations). After placing the lesion the response was measured again on the first and third post-operative days. The results are summarized in Table 2. The animals may be seen to fall into three groups according to whether the post-operative lymphopenic response was unchanged (++), diminished (+), or absent (0). Six rabbits showed what were considered to be diminished responses (249, 254, 259, 260, 261, 277). It should be mentioned that observations on rabbits 249 and 254 on the third post-operative day showed a light fall in blood lymphocytes to 90% of the initial figure 1½ hr. after the stimulus; these figures are not given in Table 2.

The lesions made in the region of the hypophysial stalk usually damaged more than one structure. In Table 2 are listed all the structures involved in the lesions. By correlating the structures damaged with the lymphopenic responses in different rabbits it is possible to make the following statements.

TABLE 2. Lymphopenic responses to an emotional stress stimulus after placing lesions in different regions of the hypothalamus or pituitary gland. The response to emotional stress is represented by the lymphocyte count at the third hour after the stimulus given as a percentage of the initial lymphocyte count

		Lymphopenic response			
Rabbit	Pre-op.	1st day Post-op.	3rd day Post-op.	Group	Lesions
246	83	54	—	++	Single mid-line. Pars distalis
247	60	67	—	++	Single mid-line. Pars distalis
249	—	93	99	+	Single mid-line. Pars distalis, intermedia, infundibular stem, tuber cinereum
250	—	96	107	0	Single mid-line. Zona tuberalis*
251	69	76	63	++	Single mid-line. Pars distalis
252	68	69	73	++	Single mid-line. Posterior part tuber cinereum
253	77	82	82	++	Single mid-line. Pars distalis, intermedia and infundibular stem*
254	58	88	106	+	Single mid-line. Zona tuberalis, anterior part
255	69	104	101	0	Single mid-line. Zona tuberalis and infundibular stem
256	81	73	60	++	Single mid-line. Central part of mammillary body
257	81	65	86	++	Single mid-line. Pars distalis and intermedia. Infundibular stem and tuber cinereum*
259	77	93	84	+	Single mid-line. Posterior part of mammillary body
260	73	93	88	+	Single mid-line. Pars distalis
261	66	118	94	+	Single mid-line. Pars distalis, pars intermedia, infundibular stem and tuber cinereum
263	84	88	82	++	Single mid-line. High in posterior part of tuber cinereum
264	67	92	70	++	Single mid-line. Posterior to optic chiasma
265	69	111	98	0	Single mid-line. Tuber cinereum, infundibular stem and pars intermedia*
266	52	101	99	0	Single mid-line. Tuber cinereum, infundibular stem, pars intermedia and pars distalis
267	77	70	75	++	Single mid-line. Infundibular stem and tuber cinereum
268	59	80	64	++	Single mid-line. Above tuber cinereum
270	70	72	63	++	Single mid-line. Infundibular stem and tuber cinereum
277	56	84	94	+	Bilateral. Posterior region of tuber cinereum*
278	55	82	83	++	Bilateral. Posterior region of tuber cinereum
279	73	121	96	0	Bilateral. Posterior region of tuber cinereum
280	60	102	102	0	Bilateral. Mammillary body*

++, represents normal lymphopenic response to emotional stress stimulus after operation; +, diminished or slight response; 0, abolition of the response. The structures involved in the lesions, to a greater or lesser extent, are listed.

* See Pl. 2, figs. 6, 11.

(*a*) *Lesions of the pars distalis.* Large lesions confined to the pars distalis (exclusive of the zona tuberalis) do not interfere with the lymphopenic response (rabbits 246, 247, 251). Two rabbits (253, 257) (Pl. 2, figs. 6, 7) in which other parts of the pituitary were involved as well as the pars distalis, showed normal lymphopenic responses. In other cases (249, 261, 266) in which the tuber cinereum was damaged as well as the pars distalis, the response was diminished or abolished. In only one animal (260) was a diminished response seen to follow a lesion confined to the pars distalis.

(*b*) *Lesions of the pars intermedia.* In no case was this structure alone damaged. In two rabbits (253, 257; see Pl. 2, figs. 6, 7), extensive damage to the pars intermedia was associated with a normal lymphopenic response; in two (249, 261) with a diminished response; and in two others (265, 266) with abolition of the response.

(*c*) *Lesions of the zona tuberalis.* This specialized region of the pars distalis was involved in three cases. In rabbit 250 (Pl. 2, fig. 8) the lesion clearly involved the whole of the zona tuberalis and the bony margin of the sella turcica, but no other structure; the lymphopenic response was abolished. In rabbit 254, the anterior region of the zona tuberalis was damaged, and the response was diminished. In the third case (rabbit 255) the zona tuberalis and the infundibular stem were extensively involved, and the response abolished.

(*d*) *Lesions of the infundibular stem.* In no case was this structure alone damaged. However, it was completely interrupted in rabbit 257 (Pl. 2, fig. 7), almost completely interrupted in rabbit 267, and damaged in rabbits 253 and 270. These four animals showed normal lymphopenic responses. In other cases in which the tuber cinereum or zona tuberalis were damaged as well as the infundibular stem, the response was diminished or abolished.

(*e*) *Lesions of the tuber cinereum.* In nine rabbits a single mid-line lesion was found to involve the posterior part of the tuber cinereum. In two animals (265, 266) the lesion extended laterally the width of the tuber cinereum in the region of the posterior part of the pars tuberalis or the posterior part of the primary plexus of the hypophysial portal vessels (Pl. 2, fig. 9). In these two rabbits the lymphopenic response was abolished. In both cases the lesion also interrupted the infundibular stem and extended into the adenohypophysis. In two other rabbits (249, 261) the lesion involved the pars distalis, pars intermedia, infundibular stem and the posterior part of the tuber cinereum. The tuberal lesion was again situated in the posterior part of the primary plexus but more localized to the mid-line. These animals showed a diminished lymphopenic response. In rabbit 257 the lesion involved the same structures but the tuberal lesion was confined to the mid-line and the lymphopenic response was normal. Rabbits 267 and 270 that had small mid-line lesions in the tuber cinereum behind the primary plexus, and rabbits 252 and 263 that had larger mid-line lesions more posteriorly, also showed normal lymphopenic responses.

In three rabbits (277, 278, 279) bilateral lesions involved the posterior part of the tuber cinereum. One animal (278) gave a normal lymphopenic response, one a diminished response (277) (Pl. 2, fig. 10) and in one (279) the response was abolished.

(*f*) *Lesions of the mammillary body.* In three animals the lesion was found to involve the mammillary body. In rabbit 259 the posterior part of this structure was damaged and the lymphopenic response found to be diminished; in rabbit 256 the lesion was confined to the mid-line part of the mammillary body and the response found normal; and in rabbit 280, a bilateral lesion completely destroyed the mammillary body without damage to surrounding structures (Pl. 2, fig. 11) and the lymphopenic response was abolished.

(*g*) *Other hypothalamic lesions.* In two rabbits that gave normal responses median lesions were found to be situated posterior to the optic chiasma (264) and above the tuber cinereum (268).

The evidence indicates that lesions of the zona tuberalis abolish the lymphopenic response, and that similar lesions in the pars distalis, pars intermedia or infundibular stem are compatible with a normal lymphopenic response. Lesions which extend transversely in the posterior region of the tuber cinereum or in the mammillary body are often associated with a loss or diminution of response.

DISCUSSION

The lymphopenic response has been found of great value in the present study in indicating anterior pituitary activity. When using this response it is essential to work without anaesthesia and with quiescent animals. For this purpose the method of electrical stimulation used was very satisfactory. Incidental emotional stress during the course of an experiment may produce a lymphopenia which repetition of the experiment shows was not due to the electric stimulation. A single positive response in an animal is not significant, whereas a single negative result is highly significant. We should also like to stress the importance of being able to repeat an experiment many times on any individual animal. This allows the elimination of many variable factors (changes in diet, external temperature, phase of sex cycle and so on), and enables definite conclusions to be drawn from the study of fewer animals than would otherwise be possible.

It has been shown that emotional stress produces a lymphopenia in the normal, but not in the hypophysectomized rabbit (Colfer *et al.* 1950). The mechanism by which the nervous system causes anterior pituitary secretion (of probably the adrenocorticotrophic hormone) and so a lymphopenia has received little attention in the past. The observation of Mikkelsen & Hutchens (1948), that electric shock therapy in man is followed by a lymphopenia 3 hr. later, made it seem likely that some part of the neural mechanism underlying the response would be excitable to electric stimulation. This is so, for localized

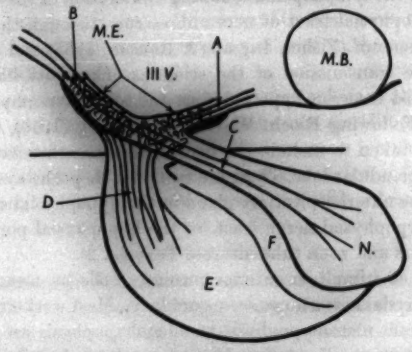
stimulation of the posterior part of the tuber cinereum or mammillary body has been found to produce a lymphopenic response similar to that following emotional stress. The evidence indicates that transverse lesions in the posterior part of the tuber cinereum or mammillary body, or lesions in the zona tuberalis of the pituitary gland, abolish the response to emotional stress. It is felt that the evidence derived from study of the lesions is not so clear cut as that derived from the study of stimulation. However, both lines of approach indicate the same conclusions.

The stimulus from hypothalamus to hypophysis does not pass by way of the cervical sympathetic system, as shown by the fact that cervical sympathectomy does not abolish the lymphopenia following stimulation of the tuber cinereum. The tubero-hypophysial tract of nerve fibres runs through the posterior wall of the tuber cinereum (Fisher, Ingram & Ranson, 1935) and is possibly concerned with the transmission of the stimulus. The most likely anatomical pathway involved between hypothalamus and adenohypophysis is the hypophysial stalk. Following Rioch, Wislocki & O'Leary (1940), the term hypophysial stalk is taken to include the neural stalk together with its sheath of portions of the glandular lobe. There are two possible pathways between hypothalamus and the anterior pituitary gland in the hypophysial stalk: either the hypothalamo-hypophysial nerve tract, or the hypophysial portal vessels contained in the pars and zona tuberalis (see Text-fig. 2).

Transmission of stimuli to anterior pituitary cells by means of the hypothalamo-hypophysial nerve fibres seems unlikely. Most workers find that nerve fibres passing from neurohypophysis to adenohypophysis are very scanty in number, if present at all. A few fibres have been described, however (see Harris, 1948*c*), as passing from the supraoptico-hypophysial tract in the median eminence to the pars and zona tuberalis, and from the infundibular stem and process to the pars intermedia. It appears unlikely that either of these sets of fibres is concerned in the lymphopenic response for the following reasons: (i) stimulation of the supraoptico-hypophysial tract in the median eminence (*B*, in Text-fig. 2) or the infundibular stem (*C*) does not evoke a lymphopenia; (ii) stimulation of the zona tuberalis (*D*) does not evoke a lymphopenia; (iii) interruption of the infundibular stem (*C*) does not prevent a lymphopenic response following emotional stress.

Transmission of stimuli to anterior pituitary cells by means of a humoral agent carried from the median eminence to the pars distalis via the hypophysial portal vessels seems probable. It has been suggested (Harris, 1944; Green & Harris, 1947) that the hypothalamus may influence anterior pituitary secretion by a two-link chain: nerve fibres passing from the hypothalamus to the median eminence where they liberate a humoral transmitter into the hypophysial portal vessels, which in turn carry the substance to the pars distalis. This theory is in accordance with the facts noted in the present work. Electrical stimulation of

the posterior part of the tuber cinereum evokes a lymphopenia, possibly through stimulation of nerve fibres which are passing to the primary plexus of the portal vessels in the median eminence. The fact that a lymphopenia is not produced by electrical stimulation of the pars distalis, pars intermedia, pars or zona tuberalis of the pituitary gland may be because the adenohypophysis lacks a secreto-motor nerve supply and is humorally controlled. Again lesions of the zona tuberalis block the lymphopenia which normally follows emotional stress (even though electrical stimulation of this structure does not evoke the response), and this may be due to the fact that all the portal vessels traverse the zona tuberalis in passing to the main part of the pars distalis (Harris, 1947*a*).



Text-fig. 2. Diagram of a sagittal section through the hypothalamus and pituitary gland of a rabbit, to illustrate the anatomy and to summarize the results obtained. *A*, posterior wall of the tuber cinereum containing the tuberohypophysial tract (stimulation evokes lymphopenia; most lesions here block lymphopenic response to emotional stress); *B*, anterior wall of the tuber cinereum containing the supraopticohypophysial tract (stimulation negative); *C*, infundibular stem (stimulation negative; lesions do not abolish lymphopenic response); *D*, zona tuberalis containing the trunks of the portal vessels and possibly some nerve fibres from the supraopticohypophysial tract (stimulation negative; lesions block lymphopenic response to emotional stress); *E*, main part of pars distalis (stimulation negative; subtotal lesions do not block lymphopenic response); *F*, pars intermedia; *M.B.*, mammillary body; *M.E.*, median eminence, surrounded by the very vascular collar of pars tuberalis, and containing the primary plexus of the hypophysial portal vessels; *N.*, neural lobe of the hypophysis; III *V.*, third ventricle.

It is of interest that the evidence indicates the hypophysial portal vessels as the pathway underlying humoral control of the secretion of gonadotrophic hormone(s) in the rabbit (Harris, 1948*b*) and in the rat (Harris, 1950). The work of Markee and his colleagues (Markee, Sawyer & Hollinshead, 1948; Sawyer, Markee & Townsend, 1949; Sawyer, Everett & Markee, 1949; Everett, Sawyer & Markee, 1949) indicates that an adrenergic substance humorally excites the secretion of gonadotrophic hormone(s), and that the action of this substance may be blocked by sympatholytic drugs, such as dibenamine. It

Figs. 1–5.

To face p. 344

Figs. 6–11.

would be of interest to see whether sympatholytic drugs also blocked the lymphopenic response to emotional stress.

SUMMARY

1. Electrical stimulation of the posterior region of the tuber cinereum or of the mammillary body, of unanaesthetized, unrestrained rabbits resulted in a lymphopenia, which was similar in time relations and magnitude to that following an emotional stress stimulus. Cervical sympathectomy did not abolish this response. Electrical stimulation of certain other regions in the hypothalamus (including the supraopticohypophysial tract), of the pars and zona tuberalis, pars distalis, pars intermedia and infundibular stem of the pituitary gland did not elicit the response.

2. The lymphopenic response which follows an emotional stress stimulus in normal rabbits was abolished by lesions in the zona tuberalis (two cases), and, in most cases, was abolished or diminished by transverse lesions in the posterior region of the tuber cinereum or in the mammillary body. Similar lesions in the pars distalis and pars intermedia, and lesions which interrupt the infundibular stem, were compatible with normal responses.

3. The conclusion is drawn that anterior pituitary secretion (of probably the adrenocorticotrophic hormone) is under neural control via the hypothalamus and the hypophysial portal vessels of the pituitary stalk.

We are grateful to Mr L. Hatton for the radiography and microphotography involved in this work, and to Mr R. R. W. Dye for valuable technical assistance and for his care of the animals. Our thanks are also due to Mr L. Herbert, of the Research Laboratories of the British Thompson-Houston Company, Ltd., Rugby, for advice on the ignitron circuit.

REFERENCES

Chaffee, E. L. & Light, R. U. (1934). *Yale J. Biol. Med.* **7**, 83.
Colfer, H. F., de Groot, J. & Harris, G. W. (1950). *J. Physiol.* **111**, 328.
Dougherty, T. F. & White, A. (1944). *Endocrinology*, **35**, 1.
Everett, J. W., Sawyer, C. H. & Markee, J. E. (1949). *Endocrinology*, **44**, 234.
Fisher, C., Ingram, W. R. & Ranson, S. W. (1935). *Arch. Neurol. Psychiat., Chicago*, **34**, 124.
Green, J. D. & Harris, G. W. (1947). *J. Endocrinol.* **5**, 136.
Harris, G. W. (1937). *Proc. Roy. Soc.* B, **122**, 374.
Harris, G. W. (1944). Thesis for M.D. degree. Cambridge University.
Harris, G. W. (1947*a*). *J. Anat., Lond.*, **81**, 343.
Harris, G. W. (1947*b*). *Philos. Trans.* B, **232**, 385.
Harris, G. W. (1948*a*). *J. Physiol.* **107**, 412.
Harris, G. W. (1948*b*). *J. Physiol.* **107**, 418.
Harris, G. W. (1948*c*). *Physiol. Rev.* **28**, 139.
Harris, G. W. (1950). *J. Physiol.* **111**, 347.
Jacobsohn, D. & Westman, A. (1940). *Acta Physiol. Scand.* **1**, 71.
Markee, J. E., Sawyer, C. H. & Hollinshead, W. H. (1948). *Rec. Progr. Horm. Res.* **2**, 117.
Mikkelsen, W. P. & Hutchens, T. T. (1948). *Endocrinology*, **42**, 394.
Rioch, D. McK., Wislocki, G. B. & O'Leary, J. L. (1940). *Res. Publ. Ass. nerv. ment. Dis.*, **20**, 3.
Sawyer, C. H., Everett, J. W. & Markee, J. E. (1949). *Endocrinology*, **44**, 218.
Sawyer, C. H., Markee, J. E. & Townsend, B. F. (1949). *Endocrinology*, **44**, 18.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photograph of an oscilloscope tracing of the pulse wave from a secondary coil (similar to the implanted coils) placed in the field of the primary coil. Duration of pulse 0·5 msec.

Fig. 2. Microphotograph of a horizontal section through the hypothalamus and surrounding tissue of rabbit 210. Note the electrode site, *E.*, situated in the anterior tuber cinereum, immediately adjacent to the anterior end of the third ventricle, III *V*. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 3. Microphotograph of a horizontal section through the hypothalamus and surrounding tissues of rabbit 273. Note the electrode site, *E.*, situated in the posterior tuber cinereum, just lateral to the third ventricle, III *V*. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 4. Microphotograph of a horizontal section through the tuber cinereum and surrounding tissues of rabbit 237. Note the electrode site, *E.*, in the region of the supraopticohypophysial tract in the anterior wall of the tuber cinereum. *I.C.*, internal carotid artery. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 5. Microphotograph of a horizontal section through the pituitary gland and surrounding tissues of rabbit 274. Note the electrode site, *E.*, in the pars distalis, *P.D.*, and adjacent to the infundibular stem, *I.S.* *I.C.*, internal carotid artery. *I.P.*, infundibular process. Section 200 μ. thick. Haemalum and eosin. ×10.

PLATE 2

Fig. 6. Microphotograph of a sagittal section through the hypothalamus and pituitary gland of rabbit 253. Blood vessels injected with indian ink. Note the lesion, *L.*, in the pars distalis, *P.D.*, and pars intermedia of the pituitary and partially involving the infundibular stem, *I.S.* *H.*, hypothalamus. *I.P.*, infundibular process. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 7. Microphotograph of a sagittal section through the hypothalamus and pituitary gland of rabbit 257. Blood vessels injected with indian ink. Note the lesion, *L.*, involving the pars distalis, *P.D.*, pars intermedia, infundibular stem and the posterior region of the tuber cinereum, *T.C.* Section 250 μ. thick. Haemalum and eosin. ×10.

Fig. 8. Microphotograph of a sagittal section through the hypothalamus and pituitary gland of rabbit 250. Blood vessels injected with indian ink. Note the lesion, *L.*, involving the zona tuberalis at the anterior pole of the pituitary gland. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 9. Microphotograph of a sagittal section through the hypothalamus and pituitary gland of rabbit 265. Blood vessels injected with indian ink. Note the lesion, *L.*, involving the posterior region of the tuber cinereum, infundibular stem and pars intermedia of the pituitary gland. *I.P.*, infundibular process. *P.D.*, pars distalis. Section 250 μ. thick. Haemalum and eosin. ×10.

Fig. 10. Microphotograph of a transverse section through the hypothalamus and pituitary gland of rabbit 277. Blood vessels injected with indian ink. Note the bilateral lesions, *L.*, extending to the floor of the hypothalamus, in the posterior part of the tuber cinereum. *N.*, third nerve. *P.*, pituitary gland. Section 200 μ. thick. Haemalum and eosin. ×10.

Fig. 11. Microphotograph of a transverse section through the hypothalamus and pituitary gland of rabbit 280. Blood vessels injected with indian ink. Note the bilateral lesion, *L.*, situated in the mammillary body. *P.*, pituitary gland. Section 200 μ. thick. Haemalum and eosin. ×10.

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OESTROUS RHYTHM. PSEUDOPREGNANCY AND THE PITUITARY STALK IN THE RAT

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Results obtained by different workers from experiments involving section of the pituitary stalk show marked discrepancies. Very varied conclusions have been drawn as to the importance of an intact pituitary stalk in relation to the functions of the anterior pituitary gland. This statement is true for the various functions of the gland that have been studied, gonadotrophic, lactogenic, thyrotrophic, adrenocorticotrophic and diabetogenic. The subject has been generally reviewed and the literature summarized (Harris, 1948*a*). Several workers have studied the effect of stalk section on the gonadotrophic functions of the rat. Richter (1933) and Brooks (1940) found this procedure produced lengthened oestrous cycles in female rats; Westman & Jacobsohn (1938*a*, *b*) and Brolin (1945) reported gonadal atrophy followed stalk section in male and female rats; whereas Dempsey & Uotila (1940) and Dempsey & Searles (1943) observed normal, lengthened or absent oestrous cycles in stalk-sectioned animals. Equally discordant results have been obtained in other forms such as the rabbit, guinea-pig and dog.

The hypophysial stalk has two main component parts, the hypothalamo-hypophysial tract of nerve fibres associated with the neurohypophysis, and the hypophysial portal vessels associated with the adenohypophysis. Regeneration of the nerve tract after section has been shown not to occur (Fisher, Ingram & Ranson, 1938), but regeneration of the portal vessels after section in the rat does occur (Harris, 1949). It is possible that the varied results obtained by different workers following pituitary stalk section may be due to different degrees of regeneration of the portal vessels. To test this hypothesis, it was decided to investigate the reproductive functions of female rats in relation to the state of the hypophysial portal vessels and the pituitary gland in the following groups of animals:

(*a*) Rats in which the pituitary stalk had been exposed but not cut.

(*b*) Rats in which the stalk had been cut.

(c) Rats in which the stalk had been cut and small foreign bodies (usually paper plates) inserted between the cut ends, in an attempt to prevent vascular regeneration.

In order to see how rapidly regeneration of the portal vessels occurred, further rats were killed at intervals of 1–10 days after stalk section.

METHODS

Adult, piebald (Lister strain) or albino, female rats of body weight 160–220 g. were used.

Operative technique. The pituitary stalk was sectioned by a temporal approach which gives a clear view of the hypothalamic region and better access than other approaches, for placing small foreign bodies between the cut ends of the stalk. Since a description of this operation has not been found in the literature, a summary of the method is given below. After anaesthetizing with ether, an inverted L-shaped incision is made in the skin (Text-fig. 1), the vertical limb of which passes close to the genal vibrissa just below the outer corner of the eye. (The terminology used here follows that of Greene, 1935.) The platysma and frontalis muscles are reflected and are retracted with the skin by thread sutures. A vertical incision is then made through the temporalis fascia cutting the temporal branch of the facial nerve but taking care to leave intact the upper zygomatic branch of the facial nerve and the superficial temporal vessels. If these latter structures are cut a troublesome conjunctivitis will later ensue. The part of the temporalis fascia posterior to the vertical incision is now removed (Text-fig. 2). The anterior border of the temporalis muscle is carefully separated from the orbital fascia and the whole muscle, down to its insertion into the coronoid process, is excised. Particular attention must be paid to that part of the muscle which is attached to the bone just posterior to the orbit. This region of the skull must be thoroughly cleaned and at the same time care taken to preserve the orbital fascia, for if this fascia is damaged haemorrhage occurs from the orbital venous sinuses. After removing the periosteum from the sagittal suture to the temporal crest and obtaining a dry field (Text-fig. 3), the side wall of the skull is removed over an area as shown in Text-fig. 4, and the dura-mater reflected. At this stage bent pins attached to threads are inserted to retract the remains of the temporalis fascia (carrying the upper zygomatic branch of the facial nerve and the superficial temporal vessels) in an antero-inferior direction. Later stages of the operation are performed under a dissecting binocular microscope (magnification $\times 20$). The brain is elevated in the region shown by the arrow in Text-fig. 4, that is at a site anterior and inferior to the jaw joint. The approach to the pituitary stalk is thus made from a point in front and lateral to the gland. This is of importance, for a clearer view is obtained with less retraction of the brain than by a strict lateral approach, and there is also less danger of injury to the jaw joint. With careful retraction of the brain in a postero-medial direction, the edge of the tentorium cerebelli and the anterior cerebral artery crossing the white optic tract appear (Text-fig. 5). In some rats a middle cerebral vein, draining into the cavernous sinus, is torn through whilst elevating the brain but the haemorrhage is easily controlled. The tip of the retractor is then placed more deeply beneath the optic tract, and by a slow rotatory movement (in which the handle of the retractor is depressed and the tip elevated) the median eminence is revealed. This structure is highly vascular and, therefore, appears pink in contrast to the surrounding brain tissue. By following the median eminence posteriorly, the pituitary stalk may be seen, passing caudally on the medial side of the internal carotid artery (Text-fig. 6). The stalk may be most easily interrupted by tearing with a small hook. During this procedure the stalk usually twists, so that the lower surface bearing the trunks of the hypophysial portal vessels comes into view, and then tears at about its mid-point. In order to section the stalk at any particular level, it is necessary to cut it with a knife. A fine curved knife is inserted above the stalk and, with a slight sawing motion, a cut is made firmly down on to bone. Some haemorrhage from the portal vessels occurs on section of the stalk, but ceases quickly and is followed by a visible congestion of the median eminence. If it is desired to prevent regeneration of the cut portal vessels, a small plate of paper (2×4 mm.) is inserted over the upper surface of the pituitary gland, beneath the

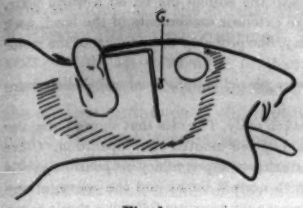


Fig. 1.

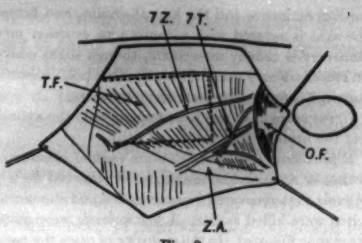


Fig. 2.

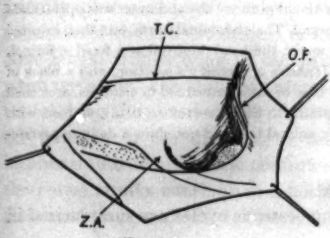
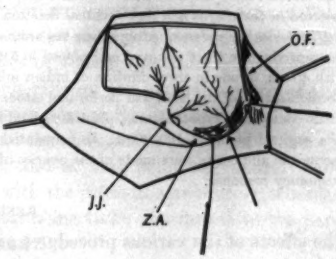


Fig. 3.



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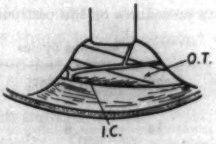


Fig. 5.

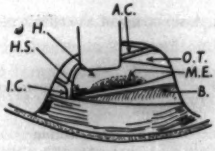


Fig. 6.

Text-fig. 1. Skin incision. *G.*, genal vibrissa.

Text-fig. 2. Exposure of temporal fascia, showing area removed (dotted line). *O.F.*, orbital fascia; *T.F.*, temporalis fascia; *Z.A.*, zygomatic arch; 7*T.*, temporal branch of facial nerve; 7*Z.*, upper zygomatic branch of facial nerve and superficial temporal vessels.

Text-fig. 3. Exposure of side wall of skull. *O.F.*, orbital fascia; *T.C.*, temporal crest; *Z.A.*, zygomatic arch.

Text-fig. 4. Lateral surface of cerebral hemisphere exposed. *J.J.*, jaw joint; *O.F.*, orbital fascia; *Z.A.*, zygomatic arch. The brain is elevated between the jaw joint and the orbit, in a postero-medial direction, as indicated by the arrow.

Text-fig. 5. Elevation of the brain has exposed the floor of the cranium, and the internal carotid artery (*I.C.*), and its branches crossing the white optic tract (*O.T.*).

Text-fig. 6. Elevation of the optic tract and adjacent brain exposes the mid-line structures. *A.C.*, anterior cerebral artery; *B.*, mid-line bone of skull; *M.E.*, median eminence; *H.S.*, hypophysial stalk with portal vessels; *H.*, hypothalamus; *I.C.*, internal carotid artery; *O.T.*, optic tract.

median eminence and the hypothalamus, and between the two internal carotid arteries. Finally, the skin is sutured with a continuous eversion suture and the animal placed on a warm plate. Recovery is usually uneventful, though slight convulsive extensor movements of the head, neck and forelimb may occur during the first post-operative day, due probably to irritation of the motor cortex. These usually cease spontaneously.

Urine output. In the later experiments the animals were placed in individual metabolism cages and the urine output measured daily.

Reproductive functions. Routine vaginal smears were taken daily. The rats that showed irregular cycles, or remained anoestrus, were observed for a period of 2–3 months after operation. Other animals that recovered regular cycles and showed a normal pseudopregnancy response to sterile coitus were killed earlier. A few animals were mated with normal bucks and the course of any pregnancy followed. In the majority of cases the pseudopregnancy response following sterile coitus was tested twice, the first time vaginal smears only being noted, and the second time a thread inserted in the uterus and any decidual reaction observed.

Histological procedures. After killing the animals with chloroform, the abdomen was opened and the reproductive tract removed and placed in 5% formol. The abdominal aorta was then injected with 40 c.c. of one in three dilution of indian ink in water, the head removed and fixed in formol. Decalcification of the head was performed either in Jenkin's or Bensley's solution, and a block of tissue containing hypothalamus, pituitary gland and base of skull embedded in celloidin, sectioned in a sagittal plane at 100–300 μ., and mounted unstained. Serial sections (10 μ.), stained with haemalum and eosin, were made of the ovaries of any animal that did not show a definite pseudopregnancy response.

RESULTS

The effects of the various procedures on the oestrous cycles are summarized in Table 1.

TABLE 1. A summary of the effects of the various operative procedures on the oestrous cycles

No. of rats	Operation	Oestrous cycles		
		Regular	Irregular	Nil
15	Blank	15	0	0
23	Simple stalk section	14	8	1
32	Stalk section with insertion of foreign body between cut ends of stalk	6	11	15
	These may be subdivided as follows:			
	(*a*) Cotton-wool plug	0	3	4
	(*b*) Celloidin-wool plug	2	1	0
	(*c*) Vaseline	1	0	0
	(*d*) Plate of beeswax	2	0	0
	(*e*) Plate of paper	1	7	11

Blank operations

The pituitary stalk was exposed in fifteen female rats, and in some was manipulated, but not cut. A pause or irregularity of the oestrous cycles occurred in some cases, but all animals eventually returned to a regular oestrous rhythm. Thirteen of the animals were tested with a sterile buck and in every case a pseudopregnancy response (and decidual reaction) ensued. One of the other two rats was mated with a normal buck and produced five living young after a pregnancy of 20 days.

The hypophysial portal vessels of these fifteen control rats were studied in serial sections, and the normal anatomy of these vessels as described by Green & Harris (1947) was confirmed. The median eminence of the rat, in surface view, forms an oval area on the base of the brain, directly anterior to the pituitary gland. Extending along the neural stalk and spreading out to cover the median eminence is the highly vascular pars tuberalis. The pars tuberalis plexus of blood vessels is supplied by small arterial twigs from the carotid artery, and from the plexus arises a rich series of vascular loops which extend into the nervous tissue of the median eminence. (These vascular loops will be referred to as the primary plexus of the portal vessels, thus following the terminology adopted by Green (1948) for analogous vessels in man.) This primary plexus is particularly profuse in the median plane of the median eminence of the rat. It drains by a series of vessels which unite to form the large portal trunks lying in the pars tuberalis on the ventral surface of the pituitary stalk. The stalk of the rat passes posteriorly in a horizontal plane. On reaching the anterior pole of the gland the stalk passes posteriorly through the foramen in the diaphragma sellae, and at this point the neural stalk inclines upwards to become continuous with the infundibular process, whereas the portal trunks continue in a horizontal plane to be distributed to the pars distalis of the gland (Pl. 1, figs. 1, 2, 5 and 6).

Simple stalk section

The term simple stalk section is used here to refer to section of the pituitary stalk without the placement of a foreign body between the cut ends. This operation has been performed on twenty-three female rats. Following operation, one rat showed no oestrous cycles, eight rats showed irregular cycles and fourteen regular cycles.

No cycles. One rat showed dioestrous vaginal smears for 115 days after operation, when it was killed. For the last 45 days a sterile buck was placed in the cage, but the presence of a mate did not result in the occurrence of any oestrous smear, and no vaginal plug was observed. After death the uterus and ovaries were found to be atrophic, the ovaries consisting of small yellow nodules in which neither ripe follicles nor corpora lutea were present.

Irregular cycles. Eight rats showed irregular, prolonged cycles following operation. Cycles of 12–15 days were common in this group and longer cycles of 30–50 days were seen occasionally. Six of these rats were tested with sterile bucks and five developed pseudopregnancy as shown by the decidual reaction following threading of the uterus. No vaginal plug was observed in the case of the sixth rat although a male was present for 49 days, during which time it showed oestrous vaginal smears on four occasions. After death this animal was observed to have only one degenerate corpus luteum in each ovary. The two rats not tested for pseudopregnancy responses were

found, post-mortem, to have ripe follicles and normal corpora lutea in their ovaries.

Regular cycles. Fourteen rats, kept alive for periods from 53 to 124 days after operation, returned to a regular oestrous rhythm. Twelve of these animals were tested with sterile males and all showed a pseudopregnancy response (and decidual reaction) following coitus. One of the remaining two rats was mated with a normal male and delivered living young after a pregnancy lasting 19 days, and the other rat was found, post-mortem, to have normal ripe follicles and corpora lutea in its ovaries.

In examining serial sections through the hypothalamus and pituitary glands of these rats, particular attention was paid to the site of the stalk section, the dimensions of the infundibular process and pars distalis, and the normality or abnormality of the primary plexus in the median eminence and of the portal trunks in the pars tuberalis. The stalk section was verified by the abnormal vascular pattern at the site of the cut, and by the atrophy of the infundibular stem and process on the pituitary side of the cut (see Pl. 1, figs. 1–8). The infundibular process was atrophic in all twenty-three animals. The pars distalis showed a central area of atrophy (necrosis) in twenty rats, which was of marked degree in eight. Of these eight rats, the oestrous cycles had been absent in one, irregular in three and regular in four; the pseudopregnancy response had been obtained in six of these rats following sterile coitus; and pregnancy with delivery of living young had been obtained in one rat which had been mated with a fertile buck.

The serial sections showed that some vascular connexion between the median eminence and pars distalis had been re-established in every case. In the rat which remained permanently anoestrus after operation, only slight regeneration had occurred, the portal trunks being represented by only a few fine capillaries. In the rats that showed irregular cycles, more regeneration was apparent, and was classified as being moderate in six animals (Pl. 1, fig. 7), and marked in two. In animals that returned to a regular oestrous rhythm the regeneration was moderate in degree in seven rats, and marked (Pl. 1, figs. 3, 4 and 8) in the other seven. The primary plexus in the median eminence appeared diminished in two animals only (one that remained permanently anoestrus, and one that returned to a regular rhythm). It may be stated then, that following pituitary stalk section in the rat, regeneration of the portal vessels occurs with facility, that in many cases large sinusoidal vessels became re-established, although in some cases smaller vessels or only a mass of fine capillaries cross the site of section, and that there is a good correlation between the return of reproductive functions and this vascular regeneration.

Stalk section plus the insertion of a foreign body between the cut ends of the stalk

In these animals the pituitary stalk was sectioned in the usual way, and then a small foreign body inserted between the cut ends of the stalk in an attempt to prevent vascular regeneration across the site of section. The effects of this procedure are summarized in Table 1.

(*a*) *Cotton-wool pledgets*

In the first seven cases minute pledgets of cotton-wool were carefully inserted on the point of a knife between the ends of the stalk.

No cycles. Four rats remained anoestrus for periods of 88, 89, 92 and 102 days, when they were killed. They were found to have atrophic uteri, and ovaries which contained degenerate corpora lutea, but no antral follicles.

Irregular cycles. Three rats showed a return of irregular cycles after operation. On testing with males, one accepted a normal male and delivered one dead and four living young on the twenty-third day of pregnancy, one mated with a sterile buck and developed functioning corpora lutea as shown by the decidual reaction, and the third refused coitus though tested with several different males (the ovaries of this animal were found to contain several large follicles, but no luteal tissue).

Serial sections through the hypothalamus and pituitary gland showed that the cotton-wool plugs swelled considerably after insertion, thereby causing trauma to the hypothalamus. In two of the rats that remained anoestrus, no vessels could be found passing between the median eminence and pars distalis, and in the other two some fine capillaries were seen to pass from the median eminence around the circumference of the wool plug, and partly through the interstices of the wool, to connect with the anterior pole of the pars distalis. In the three animals that showed irregular cycles a leash of vessels passed from the primary plexus around and partly through the wool plug to connect through the opening in the diaphragma sellae with the pars distalis. A marked central atrophy of the pars distalis had occurred in five of these seven rats with cotton-wool plugs. This atrophy was as marked in one animal that showed recurrence of cycles as in the animals that remained anoestrus.

(*b*) *Celloidin-wool pledgets*

In three rats minute pledgets of celloidin-wool were inserted between the cut ends of the stalk. Celloidin-wool, instead of cotton-wool, was chosen to facilitate the histological work (the cutting of serial sections through the hypothalamic region).

One of these three animals showed the return of irregular cycles after operation; the ovaries were found to contain normal corpora lutea and ripe

follicles. The other two rats recovered regular cycles and both showed a pseudopregnancy (decidual) reaction to sterile coitus.

Serial sections through the hypothalamus and pituitary gland showed in one rat (with regular cycles) a marked regeneration of vessels between the primary plexus and the pars distalis. These vessels ran as a compact mid-line plexus around and through the plug (Pl. 2, fig. 9) to enter the anterior pole of the pars distalis (Pl. 2, fig. 10). In the other two rats the vascular regeneration was less but still extensive. In all three animals the pars distalis showed slight central atrophy.

The use of celloidin pledgets was abandoned since they did not offer an effective barrier to vascular regeneration, and since they also swelled considerably after insertion, thus producing a hypothalamic lesion.

(c) *Vaseline*

In one rat vaseline was injected (through a fine, blunt-ended hypodermic syringe needle) between the ends of the cut stalk. In this animal regular cycles continued uninterrupted by the operation, and it was killed on the ninth post-operative day. In serial sections marked vascular regeneration was evident across the site of stalk section, the vaseline having collected under the anterior end of the stalk.

(d) *Plates of beeswax*

In two rats the attempt was made to insert small plates of beeswax between the ends of the sectioned stalk.

Both animals showed a return of regular cycles and in the one animal tested a pseudopregnancy (decidual) response followed sterile coitus.

Serial sections showed that in both cases the beeswax plates were misplaced and lying too far forward. A rich plexus of blood vessels passed around the posterior border of the plate to enter the anterior pole of the pituitary gland (Pl. 2, figs. 11, 12).

(e) *Plates of paper*

In nineteen rats small plates of paper (2 × 4 mm.) were placed across the site of stalk section. These plates were easier to manipulate, were thinner and their presence caused less damage than the other types of foreign bodies. Following this procedure, eleven rats remained anoestrus, seven showed the return of irregular cycles and one the return of regular cycles.

No cycles. Eleven rats remained anoestrus for periods varying from 58 to 106 days, when they were killed. Post-mortem, their uteri were atrophied, and histological examination of the ovaries showed atrophy of the ovarian stroma, the absence of antral follicles, and (in nine rats) the presence of some luteal tissue. Serial sections showed that in these animals the paper plates were well situated and separated the anterior pole of the pituitary gland from the hypo-

thalamus and median eminence (Pl. 2, figs. 15, 16). In five rats the pars distalis showed marked central atrophy, in five rats moderate atrophy, and in one rat none. Ten of these eleven rats showed marked permanent diabetes insipidus.

Irregular cycles. Seven rats showed irregular cycles for periods varying from 41 to 104 days after operation. Three of these animals responded to sterile coitus with a pseudopregnancy (decidual) response. Histological examination of the ovaries of the other four rats showed the presence of antral follicles and corpora lutea in the first, only luteal tissue in the second, only antral follicles in the third, and in the fourth neither luteal tissue nor antral follicles. Serial sections revealed that in these seven rats the paper plates were situated too far anteriorly to interrupt the path of vascular regeneration, and in five cases a fairly rich plexus of capillaries was found to connect the median eminence with the pars distalis. In the other two rats the capillary plexus was present but was poorly developed at one point along its course. The pars distalis was classified as moderately atrophic in six, and of normal dimensions in one rat. All seven animals showed a marked diabetes insipidus.

Regular cycles. One rat showed regular cycles for 58 days after insertion of the plate. The ovaries were found, post-mortem, to contain well-developed follicles and corpora lutea. In this animal misplacement of the paper plate had allowed a well-marked leash of capillaries to regenerate around the posterior border of the plate and re-establish vascular connexions between the pars distalis and median eminence. The pars distalis showed moderate atrophy.

A further rat, in which the stalk was sectioned and a paper plate inserted, showed the onset of a typical diabetes insipidus and on the eighth and ninth post-operative days oestrous vaginal smears. The urine output of this animal for the 7 days before operation was 4·7, 1·2, 4·0, 5·2, 6·1, 6·4, 5·4 c.c./24 hr., and for the 9 days post-operative was 90, 81, 59, 25, 8, 20, 41, 63, 80 c.c./24 hr. This rat was killed on the ninth post-operative day and it was found that the plate was situated too far anteriorly to prevent regeneration of the blood vessels and that a fine capillary plexus had regenerated across the site of stalk section (Pl. 2, figs. 13, 14).

Rate of regeneration of the hypophysial portal vessels

In a further group of eighteen rats the pituitary stalk was sectioned either with a knife or torn with a hook. These animals were killed in groups of three 1, 2, 3, 5, 7 and 10 days after operation, and the time relations of portal vessel regeneration studied.

In the three rats killed 1 day after section, a fine capillary plexus was forming across the site of section. In the rats killed on the second and third days after operation, the vessels of the regenerating plexus were enlarging in diameter. The final state reached by the vessels appears to be influenced largely by the proximity of the two cut ends of the stalk. In two animals in

which the stalk had been torn by a hook and in which the (anterior) hypothalamic end of the stalk had been intentionally left drawn forwards beneath the median eminence, the intervening gap had been bridged only by a leash of fine parallel-running capillaries. These two animals were killed on the seventh and tenth days after operation, and the pars distalis of both, showed some central atrophy. In other animals killed on the seventh and tenth days larger vessels, approximating to the normal sinusoids, were present.

DISCUSSION

The temporal approach to the pituitary stalk of the rat has several disadvantages as compared with the parapharyngeal route. It involves a wider opening of the skull, more extensive exposure of the brain, and the risk of damage to the jaw-joint, resulting in unequal growth of the incisor teeth. This approach, however, offers a better exposure of the whole hypothalamic region and allows the insertion of a plate between the cut ends of the stalk. This latter step appears to be a necessary control procedure in experiments involving stalk section in relation to anterior pituitary function. There is no doubt that regeneration of the portal vessels occurs with great facility, and in order to prevent such regeneration it is necessary to insert a plate of relatively large dimensions (2×4 mm.). This would be very difficult by the parapharyngeal approach. In the present work plates of paper were used, but in more recent experiments it has been found advantageous to use plates of waxed paper. These have the advantage that they do not soften when in contact with cerebro-spinal fluid, and thus the operator is allowed more time to manipulate them into position.

It may be seen from the results that the post-operative return of reproductive functions may be closely correlated with the amount of regeneration of the hypophysial portal vessels. This is so, although the histological appearance of the vessels gives little indication of the velocity of blood flow in them, and the appearance of the vessels may have varied according to the completeness of the indian ink injections. That stalk section in some animals was followed by varying degrees of atrophy in the central zone of the pars distalis might suggest a diminution in total blood supply to the gland. The work of Landsmeer (1947) on the vascular supply and drainage of the pituitary gland of the rat would indicate a similar conclusion. It seems unlikely, however, that reduction in the amount of anterior lobe tissue, consequent on a diminished blood supply, was responsible for the post-operative disturbances in the cycles. In many cases the normality or otherwise of the reproductive processes bore no relation to the amount of anterior lobe tissue present. Further, the indian ink injections show that the pars distalis was well vascularized in the animals in which portal vessel regeneration was prevented (see Pl. 2, fig. 15), and it is known that after incomplete hypophysectomy 30% of anterior pituitary tissue may be sufficient

to maintain normal sexual functions and as little as 10% may maintain some ovarian activity (Smith, 1932). The relationship between portal blood supply and reproductive capacity may be most easily explained on the basis that the output of gonadotrophic hormones by the pituitary is regulated by the hypothalamus via a chemical excitor (and/or inhibitor) substance transported by the portal vessels (Harris, 1944; Green & Harris, 1947). The anatomical basis for such a view is well founded in that the portal vessels are widely distributed amongst the vertebrates (Popa & Fielding, 1930; Wislocki & King, 1936; Green, 1947, 1949, 1950; Harris, 1947, 1950), and that the direction of blood flow in these vessels of the rat is from the median eminence to the pituitary gland (Green & Harris, 1949). Much experimental data have also accumulated in support of this view (Harris, 1948*a*,*b*; de Groot & Harris, 1950). Markee and his colleagues (Markee, Sawyer & Hollinshead, 1948; Sawyer, Markee & Townsend, 1949; Sawyer, Everett & Markee, 1949; Everett, Sawyer & Markee, 1949) have adduced evidence that gonadotrophic activity of the anterior pituitary gland is humorally excited by an adrenergic mechanism.

The results of the present work indicate that the *rhythm* of the oestrous state in the rat is dependent on neural, or neural and endocrine, rather than purely endocrine factors. Moore & Price (1932) suggested that oestrous rhythm was endocrine in origin, and depended on a reciprocal influence between pituitary and ovarian hormones. Later studies have been mainly concerned with the pituitary:ovarian interrelationship in more detail, and there is general agreement that oestrogens inhibit the secretion of the follicle-stimulating hormone, and increase the secretion of the luteinizing and luteotrophic (lactogenic) hormones, whilst progesterone tends to inhibit the secretion of the luteinizing hormone (see Fevold, 1944). Most workers have assumed that the effect of the ovarian hormones on the pituitary is exerted directly on the gland cells. However, Holweg & Junkmann (1932) and Holweg (1936) postulated the existence of a sexual centre in the hypothalamus which stimulates the pituitary to secrete the follicle-stimulating hormone. They suggested that the activity of this centre is depressed by oestrogens. They based their conclusions mainly on the finding that intact connexions between the pituitary and hypothalamus are necessary for the development of typical castration cells in the adenohypophysis. The evidence of Sawyer, Everett & Markee (1949) which indicates that a nervous mechanism is invoked in oestrogen-induced release of luteinizing hormone in the pregnant rat, and in the release of luteinizing hormone necessary for ovulation in the normal cyclic rat (Everett *et al.* 1949), supports this view. The conclusion drawn above that intact vascular connexions between the median eminence of the tuber cinereum and the adenohypophysis are necessary for the occurrence of oestrous cycles, again indicates the importance of the hypothalamus in normal sexual functions. Analogy might be drawn between the oestrous cycle and respiratory cycle on the grounds that the rhythmicity

of both is dependent on an intact neural mechanism, and that the activity of the nerve centres is probably governed by the chemical composition of the blood but may also be influenced by other neural (psychological) factors. The fact that in the present experiments a close correlation was seen between the post-operative return of oestrous cycles and a pseudopregnancy response to sterile coitus, would suggest that the stimulus of coitus reaches the adenohypophysis via the hypophysial portal vessels, thus reinforcing the hypothesis suggested for the rabbit (Harris, 1948*b*).

This investigation is being repeated on the monkey. The length of the pituitary stalk and of the hypophysial portal vessels, and the closer phylogenetic proximity to man, makes work on such an animal desirable.

SUMMARY

1. A temporal approach to the pituitary stalk of the rat is described. This operative route has the advantage over the parapharyngeal, that a wider access to the region is obtained, and that it allows plates of relatively large dimensions to be inserted between the cut ends of the sectioned pituitary stalk.

2. After sectioning the pituitary stalk in female rats, the recurrence of oestrous cycles and a pseudopregnancy response to sterile coitus may be correlated with regeneration of the hypophysial portal vessels. If such regeneration is prevented by the insertion of foreign bodies (usually paper plates) between the cut ends of the stalk, the animals remain anoestrus till killed (2–3 months after operation). If partial regeneration occurs, irregular and anovular cycles may be re-established.

3. Regeneration of the hypophysial portal vessels after pituitary stalk section has been observed to start within 1 day of operation.

4. These results indicate that gonadotrophic secretion by the anterior pituitary gland of the rat is dependent on the hypothalamus and on an intact vascular pathway (portal vessels) from the median eminence to the pars distalis, and that the cyclical nature of oestrus activity is neural in origin.

It gives me pleasure to acknowledge the valuable help of Mr L. Hatton in the microphotography involved in this work, and of Mr R. R. W. Dye in technical assistance, including the laborious task of taking daily vaginal smears. My thanks are due also to Mr L. G. Jarvis for help with the histological work.

REFERENCES

Brolin, S. E. (1945). *Acta Anat.* Suppl. **3**, 165.

Brooks, C. McC. (1940). *Res. Publ. Ass. nerv. ment. Dis.* **20**, 578.

Dempsey, E. W. & Searles, H. F. (1943). *Endocrinology*, **32**, 119.

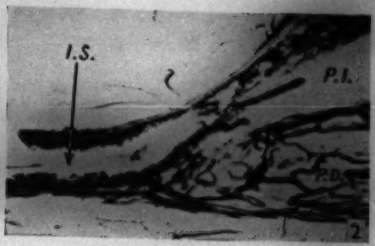
Dempsey, E. W. & Uotila, U. U. (1940). *Endocrinology*, **27**, 573.

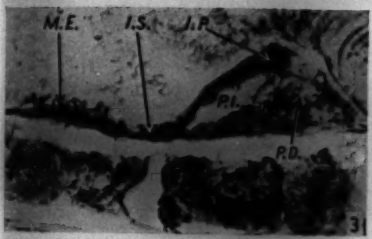
Everett, J. W., Sawyer, C. H. & Markee, J. E. (1949). *Endocrinology*, **44**, 234.

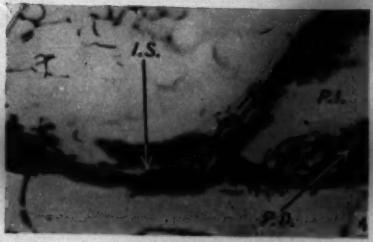
Fevold, H. L. (1944). *The Chemistry and Physiology of Hormones*, p. 152. Publication of the American Association for the Advancement of Science.

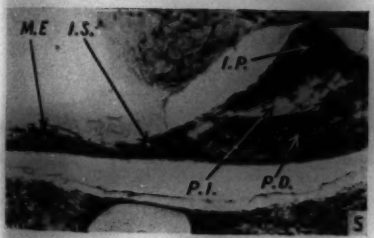
Fisher, C., Ingram, W. R. & Ranson, S. W. (1938). *Diabetes Insipidus, etc.* Ann. Arbor, Michigan: Edward Bros. Inc.

Green, J. D. (1947). *Anat. Rec.* **99**, 21.









Figs. 1–8.

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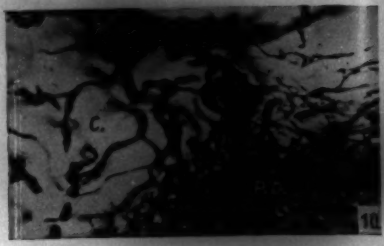

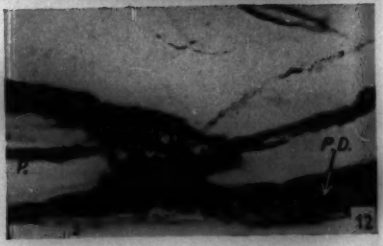

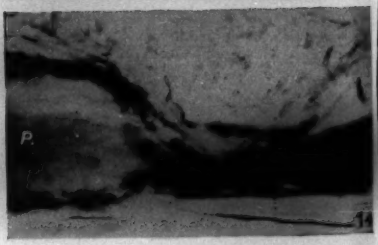

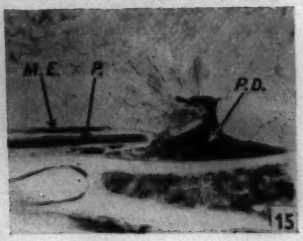

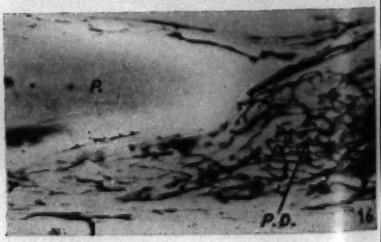

Figs. 9–16.

Green, J. D. (1948). *Anat. Rec.* **100**, 273.
Green, J. D. (1949). *J. Anat., Lond.*, **83**, 82.
Green, J. D. (1950). Personal communication.
Green, J. D. & Harris, G. W. (1947). *J. Endocrinol.* **5**, 136.
Green, J. D. & Harris, G. W. (1949). *J. Physiol.* **108**, 359.
Greene, E. C. (1935). *Trans. Amer. Philos. Soc.* **27**, 1.
Groot, J. de & Harris, G. W. (1950). *J. Physiol.* **111**, 335.
Harris, G. W. (1944). Thesis for M.D. degree. Cambridge University.
Harris, G. W. (1947). *Nature, Lond.*, **159**, 874.
Harris, G. W. (1948*a*). *Physiol. Rev.* **28**, 139.
Harris, G. W. (1948*b*). *J. Physiol.* **107**, 418.
Harris, G. W. (1949). *Nature, Lond.*, **164**, 70.
Harris, G. W. (1950). *J. Physiol.* **111**, 361.
Holweg, W. (1936). *Klin. Wschr.* ii, 1832.
Holweg, W. & Junkmann, K. (1932). *Klin. Wschr.* i, 321.
Landsmeer, J. M. F. (1947). *Het vaatstelsel van de hypophyse bij de witte rat.* Rotterdam.
Markee, J. E., Sawyer, C. H. & Hollinshead, W. H. (1948). *Rec. Progr. Horm. Res.* **2**, 117.
Moore, C. R. & Price, D. (1932). *Amer. J. Anat.* **50**, 13.
Popa, G. T. & Fielding, U. (1930). *J. Anat., Lond.*, **65**, 88.
Richter, C. P. (1933). *Amer. J. Physiol.* **106**, 80.
Sawyer, C. H., Everett, J. W. & Markee, J. E. (1949). *Endocrinology*, **44**, 218.
Sawyer, C. H., Markee, J. E. & Townsend, B. F. (1949). *Endocrinology*, **44**, 18.
Smith, P. E. (1932). *Anat. Rec.* **52**, 191.
Westman, A. & Jacobsohn, D. (1938*a*). *Acta path. microbiol. Scand.* **15**, 301.
Westman, A. & Jacobsohn, D. (1938*b*). *Acta obstet. gynec. Scand.* **18**, 109.
Wislocki, G. B. & King, L. S. (1936). *Amer. J. Anat.* **58**, 421.

EXPLANATION OF PLATES

Microphotographs of mid-line sagittal sections through the hypothalamus, pituitary gland and base of skull of rats with the vascular system perfused with indian ink. *I.S.*, infundibular stem with its sheath of vascular pars tuberalis; *I.P.*, infundibular process; *M.E.*, median eminence containing primary plexus of portal vessels; *P.D.*, pars distalis; *P.I.*, pars intermedia.

PLATE 1

Fig. 1. Rat 154. Blank operation, pituitary stalk exposed but not cut. Regular cycles and pseudopregnancy response. Section 100 μ. thick. $\times 20$.

Fig. 2. Rat 154. Pituitary stalk region (same section as in fig. 1). Note vessels passing from pars tuberalis sheath of infundibular stem into pars distalis. Section 100 μ. thick. $\times 75$.

Fig. 3. Rat 157. Stalk cut. Regular cycles and pseudopregnancy response. Note atrophy of infundibular process. Section 100 μ. thick. $\times 20$.

Fig. 4. Rat 157. Pituitary stalk region showing site of lesion (same section as in fig. 3). Note the large sinusoid passing across the site of lesion. Section 100 μ. thick. $\times 75$.

Fig. 5. Rat 148. Blank operation, pituitary stalk exposed but not cut. Note the large vessels passing from the pars tuberalis to the pars distalis. Regular cycles and pseudopregnancy response. Section 300 μ. thick. $\times 20$.

Fig. 6. Rat 148. Pituitary stalk region (same section as fig. 5). Note large mass of portal vessels passing from the pars tuberalis sheath of infundibular stem into pars distalis. Section 300 μ. thick. $\times 75$.

Fig. 7. Rat 180. Pituitary stalk region showing line of stalk section. Irregular cycles and pseudopregnancy response. Note capillary plexus crossing site of lesion. Section 100 μ. thick. $\times 75$.

Fig. 8. Rat 162. Pituitary stalk region showing line of stalk section. Regular cycles and pseudopregnancy response. Note large vessels crossing site of lesion. Section 100 μ. thick. $\times 75$.

PLATE 2

Fig. 9. Rat 217. Stalk section and pledget of celloidin-wool inserted at site of section. The pledget swelled after insertion producing thereby a marked hypothalamic lesion. The vascular plexus above the celloidin (*C.*) represents the primary plexus and trunks of the portal vessels passing around the pledget to the anterior pole of the pituitary gland; it may be clearly distinguished from a vascular reaction to the presence of a foreign body by the fact that on either side of the mid-line there is no vascular boundary between brain tissue and celloidin. Regular cycles and a pseudopregnancy response. Section 300 μ. thick. $\times 11$.

Fig. 10. Rat 217. Region of anterior pole of pituitary gland (same section as in fig. 9). Note the large vessels passing around, and through the interstices of the celloidin pledget (*C.*) to connect with the sinusoids of the pars distalis. Section 300 μ. thick. $\times 75$.

Fig. 11. Rat 231. Stalk section and a plate of beeswax (*P.*) inserted at site of section. Note the plate is situated too far anteriorly and that a vascular plexus connects the primary plexus in the median eminence around the posterior margin of the plate with the anterior pole of the pituitary gland. Regular cycles and pseudopregnancy response. Section 300 μ. thick. $\times 11$.

Fig. 12. Rat 231. Region of anterior pole of pituitary gland (same section as in fig. 11). Note the rich vascular plexus passing around the plate (*P.*) to connect with the sinusoids of the pars distalis. Section 300 μ. thick. $\times 75$.

Fig. 13. Rat 252. Stalk section and a paper plate (*P.*) inserted at site of section. Note the plate is situated too far anteriorly to prevent regeneration of the portal vessels. This animal showed the primary and permanent phases of a marked diabetes insipidus. On the eighth and ninth post-operative days it exhibited oestrous vaginal smears and it was killed on the ninth day. Section 300 μ. thick. $\times 11$.

Fig. 14. Rat 252. Region of anterior pole of pituitary gland (same section as in fig. 13). Fine capillaries may be seen in this and other sections in the series passing around the plate (*P.*), and re-establishing vascular continuity across the site of the lesion. Section 300 μ. thick. $\times 75$.

Fig. 15. Rat 244. Stalk section and a paper plate (*P.*) inserted at site of section. Note the plate (4 mm. long $\times$ 2 mm. wide) interrupts any pathway for regeneration of vessels between the primary plexus in the median eminence and the anterior pole of the pituitary gland. The anterior pole of the gland in this rat appears relatively avascular (as compared with those illustrated in figs. 5, 9, 11 and 13, which are also 300 μ. thick). It may be seen, however, that the main bulk of the pars distalis is well vascularized. This animal remained anoestrus for 79 days after operation when it was killed. Section 300 μ. thick. $\times 11$.

Fig. 16. Rat 244. Region of anterior pole of pituitary gland (same section as in fig. 15). Note the posterior margin of the paper plate (*P.*) intervenes between the anterior pole of the gland below and hypothalamus above, and that no large vessels pass around the plate to connect with the pars distalis. Section 300 μ. thick. $\times 75$.

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HYPOTHALAMO-HYPOPHYSIAL CONNEXIONS IN THE CETACEA

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The anatomical connexions between the hypothalamus and adenohypophysis are of basic importance in the problem concerning neuro-endocrine relationships. The hypophysial stalk contains nerve fibres and blood vessels and either, or both, of these structures may form part of a pathway by which the hypothalamus influences the secretion of the anterior pituitary gland. It is well known from the work of Wislocki (1929) and Wislocki & Geiling (1936) that in the Cetacea the infundibular process is separated from the pars distalis of the hypophysis by a thick dural septum. This septum limits the area of contact between the adenohypophysis and neurohypophysis to the region where the pars tuberalis lies in contact with the tuber cinereum, and therefore, any structures of functional importance to hypothalamic-adenohypophysial relationship might be expected to be concentrated in this region. Drager (1944) has described the innervation of the adenohypophysis of the porpoise. He found that a few nerve fibres pass from the hypothalamo-hypophysial tract into the pars tuberalis, but that the pars distalis was free of nerve fibres. Wislocki & Geiling (1936) comment on the vessels of the infundibular stem of the whale. They state that 'pial arteries penetrating the stalk break up locally in the neural tissue into plexiform, sinusoidal channels. From these plexuses portal veins appear to arise which conduct blood via the pars tuberalis to the anterior lobe.' It was felt that further investigation of this region in the Cetacea might give evidence of general significance.

MATERIAL AND METHODS

Both adult and foetal material was examined.

Adult material. The pituitary glands of three adult blue whales (*Balaenoptera musculus*), fixed in formol within 5 hr. of death, were dissected and photographed. Blocks of tissue from representative areas of the pars distalis of these glands were embedded in wax, sagittally sectioned at 10 μ. thickness, and stained for nerve fibres by Davenport's method (Davenport, 1930), and by Bodian's method (Bodian, 1936), using both Bayer and Winthrop brands of protargol. To control the staining procedures, pieces of brain (rabbit's brain, also formol fixed, wax embedded and sectioned 10 μ.) were stained on the same slides as the whale pituitary sections. Other sections from

these glands were stained for reticular connective tissue fibres by Gomori's method (Gomori, 1937) and again similar control tissue stained on the same slides. The fourth adult specimen was a male porpoise (*Phocoena phocoena*), weighing 62 kg. and 1·52 m. in length. This animal was injected with indian ink solution through the vertebral arteries. After fixation in formol, the hypothalamus, pituitary gland and surrounding structures were taken *en bloc*, serially sectioned in a sagittal plane at 150 μ. and stained with Biebrich scarlet.

Foetal material. The hypothalamus, pituitary gland and base of skull were removed, after formol fixation, from two foetal specimens, one, *Megaptera* (? *nodosa*), 360 mm. crown to tail tip, and the other *Balaenoptera* (? *physalus*), 290 mm. crown to tail tip. These specimens were decalcified in Jenkin's solution, embedded in celloidin, serially sectioned in a sagittal plane at 150 and 200 μ. respectively, and the sections stained with haematoxylin alone or haematoxylin and van Gieson. The brain and pituitary gland were removed in an intact dural envelope from a foetal Finback whale (*Balaenoptera physalus*), 2·44 m. long. After formol fixation a block of tissue containing hypothalamus and pituitary gland was embedded in celloidin, serially sectioned in a sagittal plane (200 μ.), and the sections stained with haematoxylin and van Gieson. The fourth foetal specimen was a female Lesser rorqual (*Balaenoptera acutorostrata*), 2·13 m. long and 101·8 kg. in weight. After attempted injection of the intracranial vessels with indian ink, the brain and pituitary gland were removed in the dural envelope and fixed in formol. A block of tissue containing the hypothalamus and pituitary gland was embedded in celloidin, serially sectioned in a sagittal plane (200 μ.), and the sections stained with haematoxylin and van Gieson, or mounted unstained.

RESULTS

Pars distalis of adult blue whales

The average dimensions of three specimens of the pars distalis of the blue whale were 3·2 cm. antero-posteriorly, 4·5 cm. transversely and 1·9 cm. supero-inferiorly, and their weights (after formol fixation) were 15·4, 20·8 and 16·6 g. From above, the glands appear kidney-shaped, the anterior pole of the gland corresponding to the hilum of the kidney. At this site is a structure closely resembling the zona tuberalis of the rabbit (Dawson, 1937; Harris, 1947). Through a foramen in the fibrous capsule which surrounds the pars distalis appears an extension of the glandular tissue which is grey in colour, semi-translucent and highly vascular as compared with the opaque white appearance of the rest of the gland. It is obvious that before removal of the brain and section of the hypophysial stalk, this region of the pars distalis was continuous with the pars tuberalis. On making a mid-line sagittal section through the gland, this tissue is seen to extend inferiorly around the anterior pole of the gland and to merge with the rest of the pars distalis. The vascular nature of this structure is again apparent on sectional view, and is well shown in Pl. 1 (figs. 3, 4). From its similarity to the zona tuberalis of the rabbit in appearance, continuity with the pars tuberalis, and relationship with the hypophysial portal vessels (see below), it is proposed to refer to this part of the adeno-hypophysis of the whale as the zona tuberalis.

The hypophysial portal vessels

Serial sections through the hypothalamus and pituitary gland of the four foetal whales show, in each case, that a connective tissue septum separates the pars distalis from the infundibular process, and that the pars tuberalis passes

upwards anterior to the septum to form a collar around the tuber cinereum (Pl. 1, figs. 1, 2). In only one of these specimens, the Lesser rorqual, are blood vessels apparent. The indian ink injection had failed to perfuse the hypophysial vessels, but these vessels are distended with blood and therefore clearly visible. Large vessels running longitudinally in the pars tuberalis (Pl. 1, fig. 2) are seen to be in continuity, at one end with large capillaries penetrating the nervous tissue of the median eminence, and at the other end with the sinusoids of the pars distalis. These vessels are clearly seen in sagittal sections through the hypothalamus and pituitary gland of the adult porpoise (Pl. 1, figs. 3, 4). In this specimen the hypophysial vessels had been successfully injected with indian ink and the highly vascular pars tuberalis is seen to pass upwards anterior to the septum and to encircle the tuber cinereum. Although the infundibular stem of the porpoise is short and not clearly demarcated from the tuber cinereum or infundibular process, it was apparent from the vascular pattern that the hypothalamus, tuber cinereum (± infundibular stem) and infundibular process form three separate vascular territories. The tuber cinereum (± infundibular stem) contains a multitude of wide, tortuous, sinusoidal vessels (Pl. 1, fig. 3) which seem to drain into the mass of vascular trunks in the pars tuberalis (the trunks of the hypophysial portal vessels). These vessels in turn pass down into the zona tuberalis where they branch and are distributed posteriorly into the sinusoids of the pars distalis. The boundaries of the zona tuberalis are well shown by the vascular pattern, as illustrated in Pl. 1, fig. 4. The fact that the vessels in the more posterior region of the pars distalis are poorly filled with ink indicates that the portal trunks were injected in a direction from above downwards.

The nerve supply and reticular fibre framework of the pars distalis of the whale

Careful examination of many sections through representative parts of the pars distalis of the blue whale (*Balaenoptera musculus*) failed to reveal any structures that could be definitely identified as nerve fibres. Particular attention was paid to sections through the upper part of the zona tuberalis, for it might be expected that any innervation the pars distalis received from the hypothalamo-hypophysial tract would be concentrated in this region. Again, no nerve fibres were seen (Pl. 2, fig. 6). The control tissue of rabbit's brain (Pl. 2, fig. 5), stained on the same slides, showed good impregnation of nerve fibres both by Davenport's and Bodian's methods. The best impregnation was obtained with the Bayer brand protargol. (This particular sample of protargol had been used in previous work to stain the hypothalamus and pituitary glands of rabbits. Very good impregnation of the fine fibres forming the hypothalamo-hypophysial tract in the hypothalamus and in the neurohypophysis had been obtained.)

The reticular fibre framework of the pars distalis of the whale impregnates well by Gomori's method. Rich plexuses of reticular fibres envelop the hypophysial portal vessels and the sinusoids of the pars distalis generally. The reticular tissue extends throughout the pars distalis, and is related to the cells of the gland in that it forms basket-like frameworks around clusters of these cells (Pl. 2, fig. 8). The control tissue of rabbit's brain, stained on the same slides, showed an absence of stained fibres except in the walls of the larger vessels penetrating the brain substance and in the pia-arachnoid on the surface of the brain (Pl. 2, fig. 7).

DISCUSSION

The hypothalamus and pituitary gland are present in all living vertebrates. Clark (1938) states: 'That this neuro-glandular mechanism comprised by the hypothalamic nuclei and the hypophysis is of fundamental importance is sufficiently attested by its remarkable uniformity throughout the vertebrate series.' Since neural control of the adenohypophysis is of basic importance, it seems likely that the mechanism by which such control is exerted remains constant throughout the vertebrates. If such an assumption is made then the evidence derived from the examination of cetacean material supports the theory that hypothalamic control over the adenohypophysis is mediated via the hypophysial portal vessels, and not by a direct nerve supply.

Most workers have found, on microscopic examination, that the anterior pituitary gland possesses a scanty nerve supply (Berkley, 1894; Gentes, 1903; Herring, 1908; Tello, 1912; Croll, 1928; Bucy, 1932; Rasmussen, 1938; Brooks & Gersh, 1941, Drager, 1944; Green & Harris, 1947; Green, 1947, 1948). These findings, and the fact that the anterior pituitary gland possesses a rich reticular fibre framework which may stain by silver methods to give the appearance of nerve fibres, puts the onus of proof on those investigators who claim to have found many nerve fibres in the gland. Such claims now need the substantiation of two control procedures: first, to stain alternate sections for nerve fibres and reticular fibres and to compare the fibre patterns disclosed; and secondly, to stain on the same slide sections from a normal gland and a 'denervated' gland (after cervical sympathectomy and pituitary stalk section) in order to demonstrate the presence of nerve fibres in the former, and their absence in the latter organ. Without these control observations, statements that many nerve fibres have been found in the pars distalis are not convincing. The recent finding of Vasquez-Lopez (1949) that many nerve fibres are present in all parts of the adenohypophysis of the rabbit should be mentioned. The pituitary glands of normal rabbits, rabbits with encephalitis and newborn animals were investigated. Some animals (it is not clear which) had been used in other experiments and inoculated with Shope papillomata or injected with carbon tetrachloride, copper salts, thyroxine or oestrogen. This worker mentions the difficulty of distinguishing connective tissue fibres from nerve fibres. It is of interest that

the best impregnation of 'nerve fibres' in the deeper parts of the pars distalis occurred in the pituitary glands of encephalitic rabbits. Vasquez-Lopez believes the explanation of this fact to be that nerve fibres take the silver best in abnormal conditions, but an alternative explanation is that the infection had caused an increase in the connective tissue element of the gland.

The literature regarding the innervation of the pituitary gland has recently been summarized (Harris, 1948). In contradiction to the scanty nerve supply, the hypophysial portal vessels or analogous vessels, form a constant and prominent feature of the pituitary glands of all animals (so far examined) from amphibians to man (Popa & Fielding, 1930; Wislocki & King, 1936; Green & Harris, 1947; Green, 1947, 1949, 1950).

The general statements made above hold true also for the pituitary gland of the Cetacea.

SUMMARY

1. The pituitary glands of four adult and four foetal whales have been examined with particular regard to the anatomical structures connecting the tuber cinereum and pars distalis.

2. The pars tuberalis extends upwards anterior to the thick septum which separates the infundibular process and pars distalis and enfolds the tuber cinereum in the way usual in other mammals. The region where the pars tuberalis merges with the pars distalis bears many points of similarity to the zona tuberalis of the rabbit, and the term zona tuberalis is applied to this region of the cetacean gland.

3. Nerve fibres were not seen in the pars distalis, though the presence of a rich reticular fibre framework was verified.

4. A prominent system of hypophysial portal vessels is associated with the pars tuberalis, linking the median eminence of the tuber cinereum and the pars distalis.

5. The functional significance of these findings is briefly discussed.

It gives me pleasure to acknowledge the kindness of Prof. H. A. Harris in allowing me to examine the foetal whale material present in the Anatomy School, Cambridge. I am also indebted to Mr D. A. Parry, Department of Zoology, and to Mr R. A. M. Case, Low Temperature Station, Cambridge, for advice and for help in obtaining material. My thanks are also due to the Dove Marine Laboratory, Cullercoats, Northumberland, for sending me the specimen of the foetal Lesser rorqual whale, and to Mr D. Canwell for his skilled help in the histological work.

REFERENCES

Berkley, H. J. (1894). *Brain*, **17**, 515.
Bodian, D. (1936). *Anat. Rec.* **65**, 89.
Brooks, C. McC. & Gersh, I. (1941). *Endocrinology*, **28**, 1.
Bucy, P. C. (1932). *The Hypophysis Cerebri.* In Penfield, W., *Cytology and Cellular Pathology of the Nervous System*, **2**, 705. New York: Paul B. Hoeber, Inc.
Clark, W. E. Le Gros (1938). *The Hypothalamus*, p. 5. Edinburgh: Oliver and Boyd.
Croll, M. M. (1928). *J. Physiol.* **66**, 316.

Davenport, H. A. (1930). *Arch. Neurol. Psychiat.* **24**, 690.
Dawson, A. B. (1937). *Anat. Rec.* **69**, 471.
Drager, G. A. (1944). *Anat. Rec.* **88**, 428.
Gentes, M. L. (1903). *C.R. Soc. Biol., Paris*, **55**, 336.
Gomori, G. (1937). *Amer. J. Path.* **13**, 993.
Green, J. D. (1947). *Anat. Rec.* **99**, 21.
Green, J. D. (1948). *Anat. Rec.* **100**, 273.
Green, J. D. (1949). *J. Anat., Lond.*, **83**, 82.
Green, J. D. (1950). Personal communication.
Green, J. D. & Harris, G. W. (1947). *J. Endocrinol.* **5**, 136.
Harris, G. W. (1947). *J. Anat., Lond.*, **81**, 343.
Harris, G. W. (1948). *Physiol. Rev.* **28**, 139.
Herring, P. T. (1908). *Quart. J. exp. Physiol.* **1**, 121.
Popa, G. T. & Fielding, U. (1930). *J. Anat., Lond.*, **65**, 88.
Raamussen, A. T. (1938). *Endocrinology*, **23**, 263.
Tello, F. (1912). *Trab. Lab. Invest. biol. Univ. Madr.* **10**, 145.
Vasquez-Lopez, E. (1949). *J. Endocrinol.* **6**, 158.
Wislocki, G. B. (1929). *Arch. Surg., Chicago*, **18**, 1403.
Wislocki, G. B. & Geiling, E. M. K. (1936). *Anat. Rec.* **66**, 17.
Wislocki, G. B. & King, L. S. (1936). *Amer. J. Anat.* **58**, 421.

EXPLANATION OF PLATES

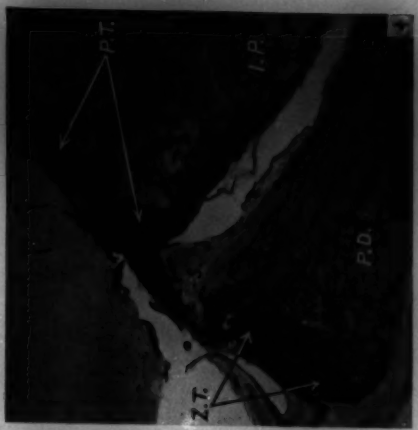
Plate 1

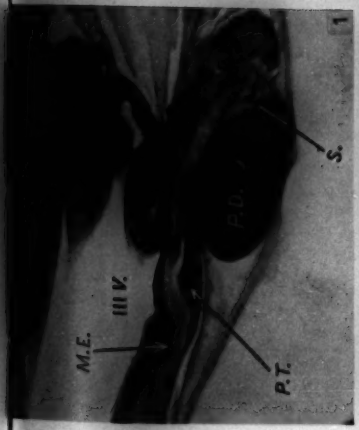
Fig. 1. Microphotograph of a mid-line sagittal section through pituitary gland and hypothalamus of foetal whale (*Balaenoptera ? physalus*). Note the pars tuberalis, *P.T.*, passing upwards anterior to the septum, *S.*, to come into intimate contact with the median eminence, *M.E.*, of the tuber cinereum. *I.P.*, infundibular process; *P.D.*, pars distalis; III *V.*, third ventricle. Section 200 μ. thick. Haematoxylin. ×17.

Fig. 2. Microphotograph of a mid-line sagittal section through the pars tuberalis and neurohypophysis of foetal whale (Lesser rorqual). *I.P.*, infundibular process; *M.E.*, median eminence of tuber cinereum; *P.T.*, pars tuberalis containing trunks of portal vessels; *S.*, septum. Section 200 μ. thick. Unstained. Blood vessels partially injected with indian ink. ×13.

Fig. 3. Microphotograph of a mid-line sagittal section through the hypothalamus and pituitary gland of the porpoise (*Phocoena phocoena*). Note the pars tuberalis, *P.T.*, rises anterior to the septum, *S.*, to encircle the tuber cinereum, and that the mass of sinusoidal capillaries in the median eminence, *M.E.*, of the tuber cinereum connect with the trunks of the portal vessels in the pars and zona tuberalis, *Z.T.*, and so with the sinusoids in the pars distalis, *P.D. I.P.*, infundibular process; *O.C.*, optic chiasma. Section 150 μ. thick. Biebrich scarlet. Blood vessels injected with indian ink. ×16.

Fig. 4. Microphotograph of a sagittal section through the lateral margin of the infundibular stem of the porpoise (*P. phocoena*). Legends as for fig. 3. Note the pars tuberalis with its vascular plexus extending around the lateral margin of the infundibular stem. The highly vascular nature of the zona tuberalis is well shown. Section 150 μ. thick. Biebrich scarlet. Blood vessels injected with indian ink. ×16.





Figs. 1–4.

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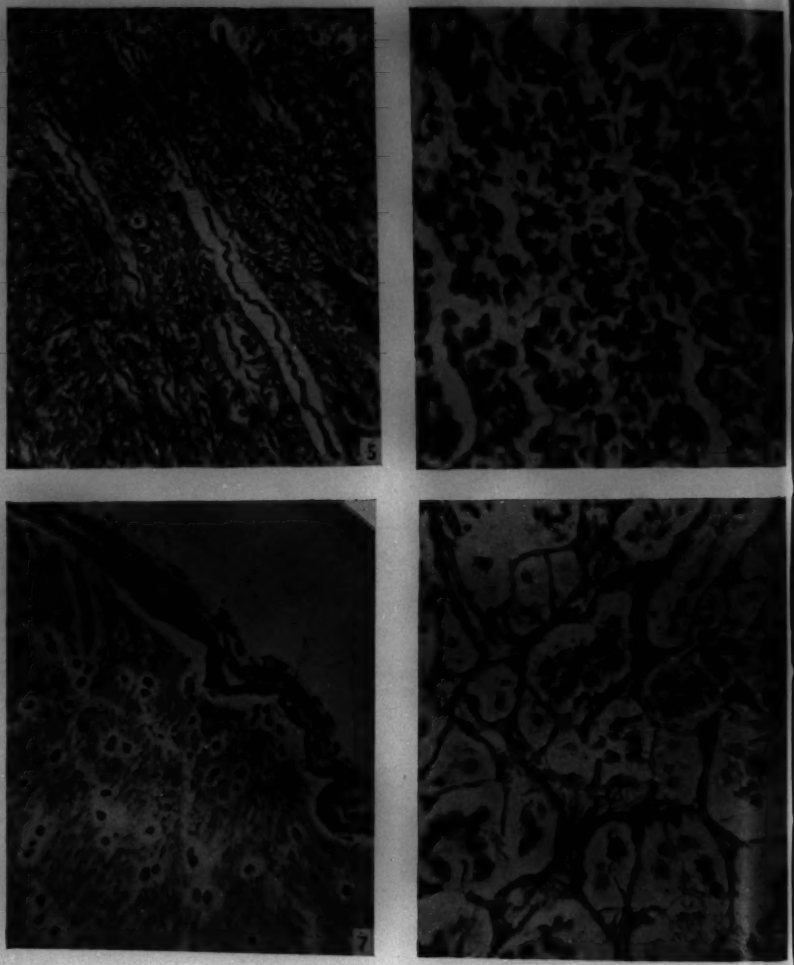

Figs. 5–8.

PLATE 2

Fig. 5. Microphotograph of a section through control tissue, rabbit's brain stained on same slide as pituitary gland shown in fig. 6. Note good impregnation of nerve fibres. Formol fixation. Section 10 μ. thick. Bodian's stain. $\times 300$.

Fig. 6. Microphotograph of a sagittal section through upper part of zona tuberalis of adenohypophysis of adult blue whale (*Balaenoptera sibbaldi*) stained on same slide as control tissue shown in fig. 5. The faint staining of vascular endothelium may be seen, but no structures that can be identified definitely as nerve fibres. Formol fixation. Section 10 μ. thick. Bodian's stain. $\times 300$.

Fig. 7. Microphotograph of a section through control tissue, rabbit's brain stained on same slide as pituitary gland shown in fig. 8. Note lack of impregnation of fibres in brain but well-stained reticular fibres in pia-arachnoid meninx on surface of brain. Formol fixation. Section 10 μ. thick. Gomori's stain. $\times 300$.

Fig. 8. Microphotograph of a sagittal section through upper part of zona tuberalis of adenohypophysis of adult blue whale (*B. sibbaldi*) stained on same slide as control tissue shown in fig. 7. The section shows the reticular fibre framework which exists around the clusters of glandular cells. Formol fixation. Section 10 μ. thick. Gomori's stain. $\times 300$.

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THE PRESSURES DEVELOPED IN ABDOMEN AND THORAX DURING THE FLACK TESTS

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Flack (1920) introduced as tests for circulatory and respiratory efficiency measurement of the maximal expiratory pressure exertable against a mercury column and of the time for which such a column could be maintained by expiratory effort at a pressure of 40 mm. Hg. These tests have become part of routine Service testing (Air Ministry, 1936), and have also been used in physiological investigations (Jokl, Cluver, Goedvolk & de Jongh, 1941), and passed into standard text-books of physiology (Bazett, 1938). Assessment of the result of the tests has been at best empirical (Air Ministry, 1936, pp. 51–2), although various hypothetical interpretations have been put upon the observed changes in pulse-rate and blood pressure (Flack & Burton, 1922; Warner & Hambly, 1925; Jokl *et al.* 1941). Warner & Hambly (1925), using gastric and oesophageal balloons connected to water manometers, attempted to measure intra-abdominal and intrathoracic pressures during the test. Since the oesophageal pressures were 18–30 mm. Hg, while the mouth pressure exceeded 40 mm., it would appear that either the glottis was closed or the oesophageal pressure was far below the intrapulmonary pressure.

Since these tests have been so widely used, it seemed desirable to measure the pressures developed in the abdominal cavity and the lungs, using optical manometers which will more accurately measure changing pressures.

METHODS

All experiments were performed upon the author, supine upon a level couch.

Pressures were recorded photographically with optical manometers, connected by water-filled tubing to a balloon in the stomach, and by air-filled tubing to an intratracheal needle of 1 mm. internal diameter and to the mouthpiece, to give the intra-abdominal, pulmonary and mouth pressures respectively. Rushmer (1946) and Mills (1950) have shown that a balloon can thus be used to record the intra-abdominal pressure. The characteristics of the manometric systems were those described by Mills (1950), that is, delays of up to 0·1 sec. in recording sudden large changes of pressure, and slight oscillation at 2–3 cyc./sec. by the intragastric manometer. The manometer used to record mouth pressure had a delay of 0·05 sec. in recording an abrupt fall of 100 mm. Hg, and showed no spontaneous oscillation. A mercury column was also connected to the mouthpiece so that the subject could observe the pressure developed.

The tests were performed with varying volumes of air in the lungs, and at the end of each expiratory effort against a closed tap the tap was opened and the subject expired maximally into a spirometer. This volume was corrected to the temperature and pressure existing in the lungs, with adjustment for the compression of residual air (assumed to be 25% of vital capacity, Hurtado & Boller, 1933; Mills, 1949) and instrumental dead space, to find the volume of air in the lungs over the residual at the peak pressure, or at 40 mm. Hg when this pressure was maintained.

RESULTS

Development of maximal pressure

Twenty-eight records were obtained of the abdominal and mouth pressures during a steady maximal expiratory effort with the cheeks supported, and another six records with the cheeks deliberately unsupported. In four and two of these experiments the intratracheal pressure was also recorded. A few deep

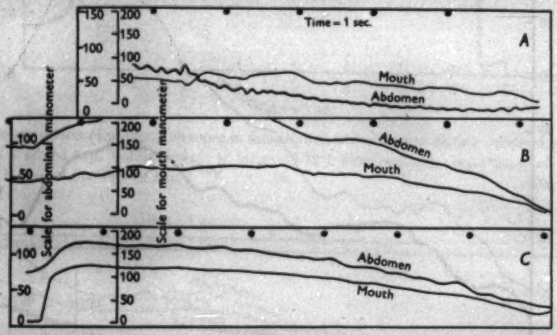

Fig. 1. Manometric record of pressure in mouthpiece and abdomen during maximal expiration against a closed tap. Pressure scales in mm. Hg. Volume of air in lungs above residual: *A*, 0·78 l.; *B*, 0·58 l.; *C*, 5 l. Read from right to left.

breaths preceded the effort, which lasted for 6–11 sec. before the tap was opened and supplemental air, 0·11–over 4 l., was expired into the spirometer. In a few experiments, owing to technical faults, this volume was not measured. The maximal pressure developed was positively correlated with the volume of air in the lungs ($r = 0{\cdot}58$, $n = 22$), although the residual variation was large. Thus when the lungs contained under 0·5 l. the peak pressure was 56–105 mm. Hg, when they contained 0·5–2 l. it was 102–140 mm. Hg, and when they held over 2 l. it was 111–158 mm. Hg. Pressures when the cheeks were unsupported are excluded from this series, as they were always lower owing to difficulty in retaining the mouthpiece with the cheeks expanded and high air pressures in the mouth.

When the cheeks were unsupported the mouth pressure rose initially, in three experiments, above the abdominal pressure. In Fig. 1*A* the excess is for a time as much as 40 mm. Hg. In three other experiments the cheeks were,

however, of no apparent assistance, and pressure developed by the cheeks was never long maintained by them, as the abdominal pressure soon rose to near the mouth pressure.

Apart from this use of the cheeks, the mouth and intra-abdominal pressures rose more or less in parallel, as is seen in Figs. 1–4. When the volume of the air in the lungs was low, as in Figs. 1*B*, 2 and 4*A*, *B*, the abdominal was greater than the mouth pressure, and when there was little air in the lungs above the residual the abdominal pressure once rose over 157 mm. Hg in developing

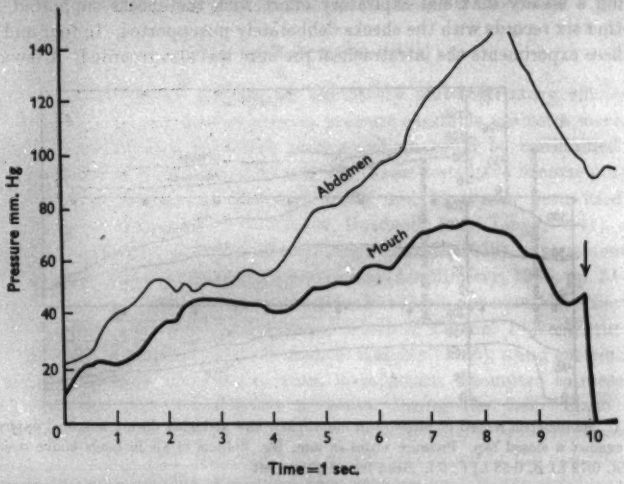

Fig. 2. Reconstruction of pressure changes in mouthpiece and abdomen during maximal expiration against a closed tap. Volume of air in lungs=0·31 l. over residual. Read from left to right. Arrow indicates opening of tap.

a mouth pressure of 56 mm. Hg. With air volumes of 1 or 2 l. above residual the two pressures were close together, except that at high pressures, over 100 mm. Hg, the abdominal rose well above the mouth pressure. With large volumes of air in the lungs, as in Figs. 3 and 1*C*, the mouth pressure equalled and often exceeded the abdominal pressure. When the tracheal pressure was recorded, as in Fig. 4*A*, *B*, it corresponded closely to the mouth pressure, except that on relaxation the mouth pressure fell before the tracheal or abdominal, and in the final expiration into the spirometer, when the mouth pressure was zero and the abdominal pressure high, the tracheal pressure rose twice to 43 and 28 mm. Hg, indicating considerable resistance to the rapid passage of air.

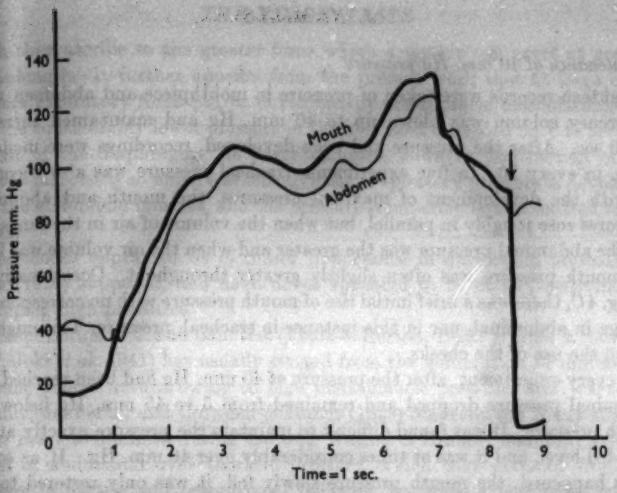

Fig. 3. Reconstruction of pressure changes in mouthpiece and abdomen during maximal expiration against a closed tap. Volume of air in lungs = 3·72 l. over residual. Read from left to right. Arrow indicates opening of tap.

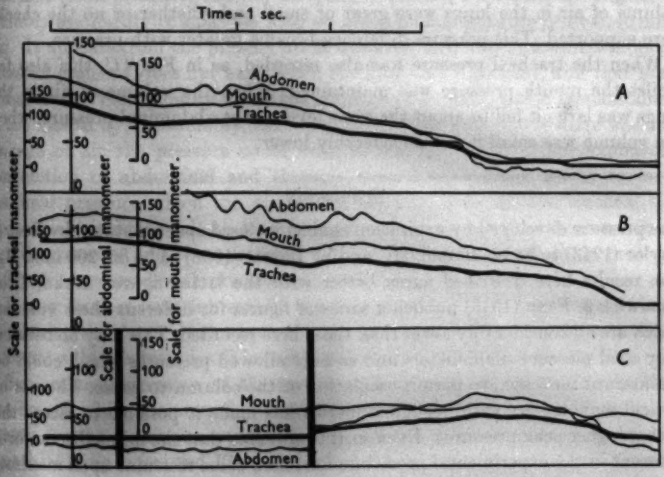

Fig. 4. Manometric records of pressures in mouthpiece, trachea and abdomen during expiration against a closed tap, *A* and *B*, maximally, *C*, trying to maintain mouth pressure at 40 mm. Hg. Pressure scales in mm. Hg. Volume of air in lungs above residual: *A*, 0·85 l.; *B*, 0·60 l.; *C*, 0·50 l. In *C* there are two intervals of 8 sec. each. Read from right to left. Oscillations of abdominal manometer in *A* and *B* are probably of instrumental origin.

Maintenance of 40 mm. Hg pressure

Eighteen records were taken of pressure in mouthpiece and abdomen when a mercury column was blown up to 40 mm. Hg and maintained there for 30–50 sec. After the pressure had been developed, recordings were made for 2 sec. in every 10. In five experiments tracheal pressure was also recorded. As with the development of maximal pressures, the mouth and abdominal pressures rose roughly in parallel, but when the volume of air in the lungs was low the abdominal pressure was the greater and when the air volume was large the mouth pressure was often slightly greater throughout. Occasionally, as in Fig. 4*C*, there was a brief initial rise of mouth pressure with no corresponding change in abdominal, nor in this instance in tracheal, pressure. This might be due to the use of the cheeks.

In every experiment, after the pressure of 40 mm. Hg had been reached, the abdominal pressure dropped and remained from 5 to 45 mm. Hg below the mouth pressure. It was found difficult to maintain the pressure exactly at the intended level, and it was at times considerably over 40 mm. Hg. If, as sometimes happened, the mouth pressure slowly fell, it was only restored to the correct level by a rise of abdominal pressure to a higher level, but apart from such occasional temporary rises the abdominal pressure always remained below the mouth pressure throughout the rest of the experiment, whether the volume of air in the lungs were great or small and whether or no the cheeks were supported. This pressure difference became greater with practice.

When the tracheal pressure was also recorded, as in Fig. 4*C*, this also fell whilst the mouth pressure was maintained. When the volume of air in the lungs was large it fell to about the same level as the abdominal pressure, when the volume was small it fell considerably lower.

DISCUSSION

The pressure developed by expiration against a closed space is stated by Best & Taylor (1943) to be 10–40 mm. Hg, and by Bazett (1938) to be 80–200 mm. Hg. The results here described agree better with the latter figure. Rahn, Otis, Chadwick & Fenn (1946) publish a series of figures for different chest volumes which are all considerably lower than those here recorded. This may be because they used mercury manometers and so only allowed pressures which could be maintained for 3 sec., to permit oscillation of the column to cease. The use of optical manometers with negligible inertia has made it possible to record the rather higher peak pressures. Even so, it is probable that the minor discomforts inherent in the experimental procedure exercised some restraint upon maximal efforts, for in preliminary practice pressures of over 200 mm. Hg have several times been developed. Rahn *et al.* (1946) also found that the maximal expiratory pressure increased with increasing volume of air in the lungs, a phenomenon

which they ascribe to the greater force which a muscle can exert at greater initial length. It further appears from the present work that at large chest volumes expiratory force can be developed by the thoracic musculature, and that the considerably lower pressure in the abdomen is needed only to prevent descent of the diaphragm. At lower volumes, however, contraction of the thoracic muscles is so much less effective that expiratory pressure is developed with the abdominal muscles, and the pressure is imperfectly transmitted to the thorax.

The cheeks appear to be of minor importance in developing pressure, as Flack (1920) supposed, and have never appeared to be of any assistance in developing high pressures.

Interpretation of the 40 mm. test (Flack & Burton, 1922; Warner & Hambly, 1925; Jokl *et al.* 1941) has usually started from the assumption of diminished systemic venous return owing to high intrathoracic pressure, and Warner & Hambly, Jokl *et al.* and Crowden & Harris (1929) observed the X-ray shadow of the heart to diminish during the test. Warner & Hambly maintain that the excess of abdominal over thoracic pressure, which they deduced from the pressure in gastric and oesophageal balloons, promotes a continued venous return. Flack & Burton, however, hold that large abdominal pooling in the unfit causes an unduly high venous return, with consequent changes in pulse-rate and blood pressure. The low oesophageal pressures recorded by Warner & Hambly are hardly compatible with an intrathoracic pressure of over 40 mm. Hg. It appears from the present work, however, that this abdominal-thoracic pressure gradient does not exist when the lungs are expanded, and that both pressures fall considerably below the pressure of 40 mm., which is maintained in the mouth by, presumably, closure of the glottis; although if there was any leakage of air the pressure could only be restored by a further expiratory contraction of abdominal and thoracic musculature. There seems to be a maximal pressure which the glottis can support, since neither tracheal nor abdominal pressures fell to their normal values during the maintenance of mouth pressure, and there is some indication that the supportable pressure difference increased with practice.

It may be that some subjects do not learn the trick of closing the glottis and so relaxing the chest. If this is so, the test becomes even less satisfactory, since different subjects tested will be subjected to very different stresses.

Subjective impression of the test is that it measures the ability to hold the breath while the attention is concentrated elsewhere. In fact, published figures for the maximum endurance time are not very far short of the simple breath-holding time, and Flack & Burton (1922) found that it is increased or decreased by those chemical factors which alter the breath-holding time, tensions of oxygen and carbon dioxide in the lungs. Hambly, Pembrey & Warner (1925) divided sixty-three subjects into six groups of diminishing physical

fitness, and found no significant differences in their endurance times in the 40 mm. test. They suggested that it might be in part a test of sensitiveness to discomfort.

If the vascular response to the test has diagnostic or prognostic significance, it is empirical and rests upon no known physiological adaptation to any determined stress.

Since air at a positive pressure of 34 mm. Hg can be rebreathed with comfort for 5 min. (Mills, 1949), the circulation must be able to adapt itself to overcome such positive intrapulmonary pressures, and since the 40 mm. test usually lasts little over 1 min. it is most improbable that it is terminated by any inability of the circulation to continue.

The test of maximal pressure exertable should be of much more value in assessing the ability of the circulation to withstand positive intrathoracic pressures such as are supposed (Jokl *et al.* 1941) to arise commonly during exercise. If the lungs are fairly full, the cavities of thorax and abdomen are subjected to a pressure close to that recorded in the mouth. The stress is one which the circulation is eventually unable to overcome, and the effort generally terminates when the subject is on the verge of unconsciousness. It is well known that a little extra pressure applied to the thorax causes consciousness to be lost. A sudden headache about 5 sec. after relaxation perhaps indicates a surge of released blood reaching the head. The briefer duration of this test also means that gaseous changes in the lungs will not be a limiting factor.

SUMMARY

1. The mouth, tracheal, and intra-abdominal (intragastric) pressures have been recorded with optical manometers, free of inertia, during expiration against a closed tap, either maximally or maintaining the pressure at about 40 mm. Hg.

2. When the chest is fairly full, expiratory pressure is developed by the thoracic musculature, and the abdominal pressure is lower. When the chest is fairly empty, expiratory pressure is developed by the abdominal musculature, and the abdominal may considerably exceed the mouth pressure. Higher pressures are developed with the chest full.

3. Mouth pressure can be maintained above 40 mm. Hg with the tracheal and abdominal pressures considerably lower, whether the chest be full or empty and whether or no the cheeks be supported. The glottis is presumably closed.

4. Interpretations of the Flack 40 mm. Hg test in terms of high intrathoracic pressures are of doubtful validity.

My thanks are due to Mr K. F. Wilsdon for the insertion of a tracheal needle, and to Mr S. Langford for technical assistance.

REFERENCES

Air Ministry (1936). *The Medical Examination for Fitness for Flying.* Air Publication 130, 4th ed. London: H.M. Stationery Office.

Bassett, H. C. (1938). In Bard, P., *Macleod's Physiology in Modern Medicine*, 8th ed. p. 413. Cambridge: Henry Kimpton.

Best, C. H. & Taylor, N. B. (1943). *The Physiological Basis of Medical Practice*, 3rd ed. p. 497. London: Baillière, Tindall and Cox.

Crowden, G. P. & Harris, H. A. (1929). *Brit. med. J.* **1**, 439.

Flack, M. (1920). Tests for flying efficiency and flying strain. *M.R.C. Rep.* **53**, 98.

Flack, M. & Burton, H. L. (1922). *J. Physiol.* **56**, 50*P*.

Hambly, W. D., Pembrey, M. S. & Warner, E. C. (1925). *Guy's Hosp. Rep.* **75**, 388.

Hurtado, A. & Boller, C. (1933). *J. clin. Invest.* **12**, 793.

Jokl, E., Cluver, E. H., Goedvolk, C. & de Jongh, T. W. (1941). *Publ. S. Afr. Inst. med. Res.* Reprint 303.

Mills, J. N. (1949). *J. Physiol.* **110**, 207.

Mills, J. N. (1950). *J. Physiol.* **111**, 376.

Rahn, H., Otis, A. B., Chadwick, L. E. & Fenn, W. O. (1946). *Amer. J. Physiol.* **146**, 161.

Rushmer, R. F. (1946). *Amer. J. Physiol.* **147**, 242.

Warner, E. C. & Hambly, W. D. (1925). *Guy's Hosp. Rep.* **75**, 286.

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THE NATURE OF THE LIMITATION OF MAXIMAL INSPIRATORY AND EXPIRATORY EFFORTS

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The random variation in successive determinations of the vital capacity is mainly due to variability of the end-inspiratory point, although subjectively the limit of inspiratory seems much sharper than that of expiratory ability (Mills, 1949). The mode of limitation of maximal respiratory movements thus deserves study. This has been attempted by measuring the intra-abdominal pressure, by recording action potentials from muscles of the abdominal wall, and by observation of the glottis.

METHODS AND SUBJECTS

Fourteen healthy male subjects and one female subject were examined, supine on a level couch.

Intra-abdominal pressure was recorded from a partly filled balloon in the stomach, connected to an optical manometer recording on photographic paper. Preliminary trials with the balloon in the rectum (Rushmer, 1946) were less successful as the balloon appeared to become kinked at times and failed to record known rises of pressure; on one occasion peristaltic waves of amplitude 30 mm. Hg were observed, at a frequency of about 2 per min. With the gastric balloon no such difficulty was encountered except when the subject was visibly retching. When rectal and gastric balloons were used simultaneously the pressures were the same to within 3 mm. Hg.

The balloon was connected to Ryle's or pressure tubing as far as the mouth, thence by lead tubing to the manometer. All tubing was completely filled with water.

The fidelity of the manometer was examined by enclosing the balloon in a jar of air at a pressure of 40–120 mm. Hg above ambient, and suddenly releasing the pressure. The photographic pressure record fell to zero within 0·1 sec., but continued to oscillate detectably for 1–1½ sec. at 2–3 cyc./sec.

Calibration of the manometer against a mercury column was frequently performed.

Deductions about the pressure exerted upon the balloon within the stomach depend upon its position, which was assumed to be 8 cm. above the surface of the couch. Errors of a few cm. in this height will have little effect. The balloon is thus presumed to be about 10 cm. below the anterior abdominal wall, and the hydrostatic pressure of overlying viscera will account for a considerable fraction of the resting pressure, of about 10 mm. Hg, leaving only a very small contribution for tone in the gastric and abdominal musculature. It is thus probable that the recorded pressure in the balloon was very close to the intra-abdominal pressure in this region. Rushmer (1946), using a rectal balloon in dogs and a direct record of intra-abdominal pressure, showed that the balloon gave an accurate reading. The gastric balloon has here been shown to give usually the same pressure as a rectal one, but to be more reliable owing to the absence of peristalsis, and the optical manometer will give a more accurate record of rapidly changing pressures than the liquid manometers used by Rushmer.

Pressure inside the trachea was recorded from a similar, but air-filled, manometer connected to a needle of internal diameter 1 mm. inserted in the trachea. When air in the manometer connexions was raised to pressures of 50, 100 and 150 mm. Hg above atmospheric and then allowed to escape to the room through the needle used in the trachea, the pressure record fell to zero within 0·10, 0·13 and 0·15 sec., and showed no subsequent oscillation. Any delay in recording pressure changes is therefore trifling.

Respiration was recorded with a Benedict type spirometer, whose movement caused a small light source to travel across the camera.

Observations of the vocal cords were made by Mr K. F. Wilsdon by indirect laryngoscopy.

Action potentials were recorded by Dr W. A. H. Rushton from the usual type of concentric electrodes inserted into the abdominal muscles. The subject was earthed through the mouth, and the hypodermic needle and central wire led to the input of a differential amplifier. A small coupling condenser was interposed between amplifier and cathode-ray output stage (CR = 5 msec.) to pass the muscle spikes and restrain the swings of base-line due to electrode movement, etc. A simultaneous respiratory tracing was obtained from a Krogh spirometer connected to a light source.

RESULTS

Abdominal pressure

Abdominal pressure records were obtained from six male and one female subjects.

Upon maximal expiration the pattern of behaviour was remarkably uniform. The abdominal pressure rose very little until expiration was nearly complete, when it rose sharply as the last small volume of air was expired, as the records

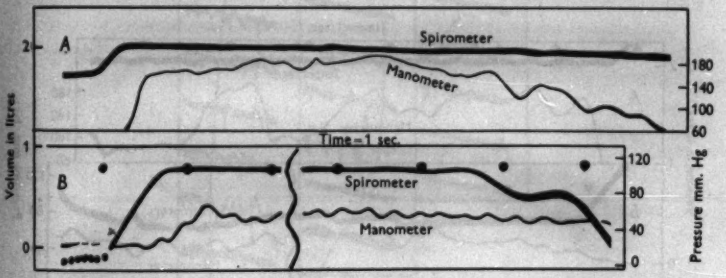

Fig. 1. Spirometer movement and abdominal pressure during maximal expiration. Subject *A*, J. N. M.; *B*, J. C. L. R. A gap of 4 sec. occurs in *B*. Inspiration downwards. Read from right to left.

in Fig. 1 show. Over 5–10 sec. while the abdominal pressure was high an extra 100–400 c.c. of air was expired. Only very rarely did the spirometer stop moving for a brief period. The peak pressures reached are shown in Table 1, and were very variable. The peak pressure on coughing was usually comparable, but varied even more with the vigour of the cough.

Upon maximal inspiration the abdominal pressure also rose, and often to a high level; but the spirometer either stopped moving completely for up to 12 sec. except for oscillations of instrumental origin, as in Fig. 2*A*, or it moved

in a series of irregular jerks separated by stationary periods, as in Fig. 2*B*. The subject appeared to be making either a sustained effort or a series of spasmodic ones. The highest abdominal pressures were always recorded when the spirometer was stationary. If the subject relaxed a little and then made a second attempt, the abdominal pressure fell and only rose again when the spirometer had once more come to rest. It appeared that high abdominal pressures were associated with unsuccessful attempts to increase inspiration.

TABLE 1. Peak intra-abdominal pressures in mm. Hg during maximal respiratory movements

	Maximal expiration		Maximal inspiration		Coughing	
Subject	No. of observations	Peak pressures	No. of observations	Peak pressures	No. of observations	Peak pressures
J. N. M.	29	62–183*	43	37–207*	6	122–191
J. H. W.	4	70–79	4	43–55	2	56–65
C. J. P.	8	57–106	8	66–74	3	124–over 133
J. C. L. R.	3	74–77	3	69–76	—	—
R. J. V.	3	94–121	3	70–74	5	58–69
W. H. C.	2	92–97	2	28–34	2	48–92
J. R. J. M.	3	64–82	3	24–41	4	76–82

* Perhaps higher, as peak pressure was often outside the recorded range.

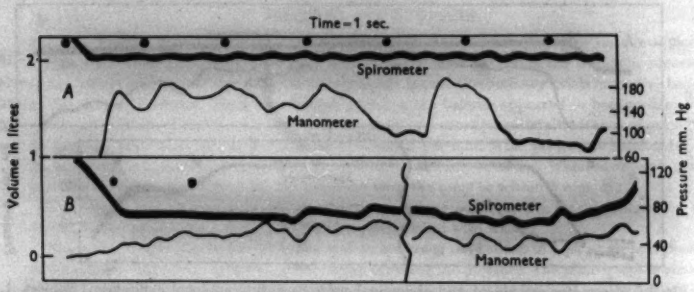

Fig. 2. Spirometer movement and abdominal pressure during maximal inspiration. Subject *A*, J. N. M.; *B*, J. C. L. R. A gap of 4 sec. occurs in *B*. Inspiration downwards. Read from right to left.

Four records were obtained upon J. N. M. of the tracheal pressure upon maximal inspiration. It was found that while the spirometer was stationary at the end of inspiration and the abdominal pressure exceeded 143 mm. Hg, the tracheal pressure was maintained at 60–70 mm. with occasional brief rises to 80–90 mm.

The high pressures recorded for J. N. M. with all procedures were probably due to frequent practice and to familiarity with the discomforts involved.

Abdominal electromyograms

The needle electrode was inserted into either obliquus externus or rectus abdominis. A satisfactory position was confirmed by observing abundant action potentials when the subject strained. Sweeps lasting about 0·16 sec. were photographed on moving paper at intervals of 0·6–0·7 sec., with a continuous spirometer record. Records of maximal expiration, maximal inspiration and coughing were made on ten subjects. Discharge always occurred

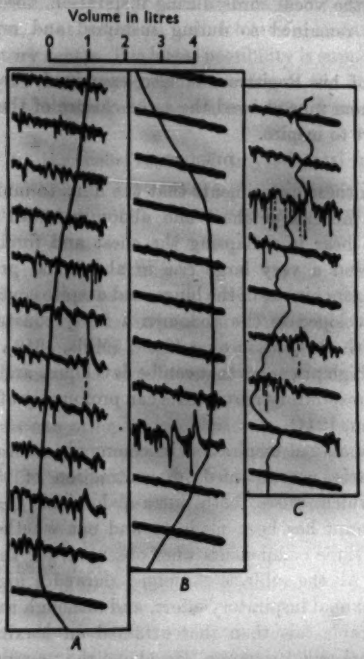

Fig. 3. Electromyographic sweeps of 160 msec., at 600 msec. intervals, from obliquus externus abdominis, with continuous spirometer tracing. *A*, maximal expiration; *B*, maximal inspiration; *C*, coughing. Expiration to left. Read from top to bottom and left to right.

during maximal expiration and coughing, as might be expected. In addition, seven subjects, including those three upon whom abdominal pressures had also been recorded, showed potentials during maximal inspiratory effort, when the movement of the spirometer had ceased. These were usually fewer than during maximal expiration, either in duration as in Fig. 3, or in frequency. In one

record spikes were observed during the brief inspiratory phase of a cough. It was not easy to pick out recognizable units firing at a steady frequency, so no further analysis of the records was attempted.

Behaviour of vocal cords

Mr K. F. Wilsdon examined the glottis of J. N. M., upon whom a rise of pressure in the abdomen and action potentials in the abdominal wall had been recorded regularly during maximal inspiration, and observed that, after the wide abduction of the vocal cords during inspiration, the cords were closely approximated and remained so during sustained and repeated inspiratory efforts.

With the help of his Registrar he also examined twenty-three hospital out-patients, of whom nine showed the same closure of the glottis at the end of a sustained effort to inspire.

DISCUSSION

It seems clear from these experiments that the limit to maximal expiration is reached when the muscles of chest and abdominal wall are bringing their maximum force to bear in collapsing the chest and forcing the diaphragm upwards. At the end a very large rise in abdominal pressure only forces a small amount of extra air out of the lungs, and despite considerable variations in the pressure developed in the abdomen a fairly constant end-expiratory level is reached, with a variation of ± 40 c.c. (Mills, 1949). The only point of interest is the very high pressures that can be developed, and it is not surprising that such powerful respiratory movements can profoundly affect the circulation (Woodbury & Abreu, 1944).

It appears that maximal inspiration is, commonly at least, terminated by closure of the glottis, accompanied by contraction of the muscles of the abdominal wall which raises both intra-abdominal and intrapulmonary pressures. No attempt has been made to find out whether this response is universal, and the three components were observed, for the most part, upon different subjects. All the subjects examined showed a high intra-abdominal pressure during maximal inspiratory effort, and although the pressure reached was often considerably less than that attained on maximal expiration the lowest pressures were still 10–30 mm. Hg above the pressure recorded during quiet breathing.

The absence of recorded action potentials in the abdominal musculature in three subjects might indicate either absence of muscular activity in those subjects, or activity of relatively few units, none of which was picked up by the electrodes. The failure of the vocal cords to approximate in some subjects might likewise be due to a different behaviour by those subjects, or to an inability to make maximal inspiratory movements when subjected to the moderate discomfort of traction on the tongue.

This involuntary closure of the glottis accounts simply for the variability of volume of maximal inspirations together with the subjective feeling of a very definite end-point (Mills, 1949). It also accords well with the very usual form of tracing of a vital capacity determination on a moving drum, when expiration appears to approach a final volume asymptotically whilst inspiration reaches a definite end-point or ends with a series of irregular jerky movements. When the glottis has once closed it seems that more air can only be inspired by relaxing, usually letting a little air out, and trying again.

This paradoxical end-inspiratory phenomenon might be a reflex consequence of pulmonary inflation, or it might be a direct consequence of excessive discharge by the inspiratory centre. The latter possibility is supported by observations on two subjects of electromyographic activity in the abdominal wall on attempting to inspire against closed mouth and nose from the normal end-expiratory position.

If one subscribes to the belief that all physiological phenomena have functional significance, this might be sought in protection of the lungs from over-distension, or in the promotion of increased venous return to the right side of the heart.

SUMMARY

1. A high intra-abdominal pressure is developed at the end of maximal inspiratory as well as of maximal expiratory efforts. Intratracheal pressure also rises at the end of a maximal inspiratory effort.

2. The vocal cords are often closely approximated during final efforts at inspiration.

3. Action potentials can often be recorded from muscles of the abdominal wall in maximal inspiration, as well as in maximal expiration and coughing.

4. It is concluded that maximal inspiration is usually terminated by closure of the glottis and contraction of the muscles of the abdominal wall.

My thanks are due to Mr K. F. Wilsdon for laryngeal observations and for the insertion of a needle in the trachea, to Dr W. A. H. Rushton for recording the electromyograms, to all those who have acted as subjects, and to Mr S. Langford for technical assistance.

Note added in proof. Moritz (1895) recorded upon himself a rise of 51 cm. H_2O in the intra-abdominal (intragastric) pressure on maximal inspiration.

REFERENCES

Mills, J. N. (1949). *J. Physiol.* **110**, 76.
Moritz (1895). *Z. Biol.* **32**, 336.
Rushmer, R. F. (1946). *Amer. J. Physiol.* **147**, 242.
Woodbury, R. A. & Abreu, B. E. (1944). *Amer. J. Physiol.* **142**, 721.

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POTASSIUM AND NEUROMUSCULAR TRANSMISSION

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In the course of experiments made in another connexion, it was observed that isolated preparations of the rat diaphragm which had been denervated were more sensitive to the depressant action of extra potassium in the Ringer solution than normal preparations. This has now been studied in detail.

METHODS

The methods were those described by Hajdu & McDowall (1949) with the modification that in many instances the muscle was stimulated directly while totally immersed in Krebs's solution, the formula of which has already been given (Hajdu & McDowall, 1949). To obtain the following results the temperature must be kept strictly at 38° C. for reasons given below. The nerve was stimulated with currents of 200 μsec. duration at 25 V., the muscle directly by currents of 2000 μsec. at 75 V. delivered by an electronic rectangular wave stimulator. The relatively large currents were used because of the total immersion of the nerve and muscle in the fluid. This is found to give the most constant results and avoids any effects of bubbles of oxygen, but calculation based on the responses of a curarized muscle with the bath empty and full indicates that only about one-tenth of this current passes through the muscle.

When desired, denervation of one side of the diaphragm was carried out by section of the phrenic nerve in the neck under ether anaesthesia and checked by the finding after 10 days of no response to nerve stimulation, and a contracture response to acetylcholine which was not present in normal innervated diaphragms (Gasser & Dale, 1926; Dale & Gaddum, 1930).

RESULTS

A small increase in the potassium concentration of the bath fluid causes an increase of the contraction of the preparation stimulated through its nerve with single maximal shocks. Larger doses cause an initial augmentation followed by a depression of the twitch. Thus, if the potassium concentration of the bath fluid is increased from 5·92 m.mol. (i.e. the concentration in Krebs's solution) to 7 m.mol., an augmentation occurs and a depression with 13·72 m.mol. In a denervated preparation, depression is brought about by 12·64 m.mol. and the early augmentary effect is slight (Fig. 1). This may seem a very small increase in sensitivity, but it has become apparent that the muscle is extremely

sensitive to minute changes in the concentration of potassium in and around this amount, while the response is materially affected by temperature (see below).

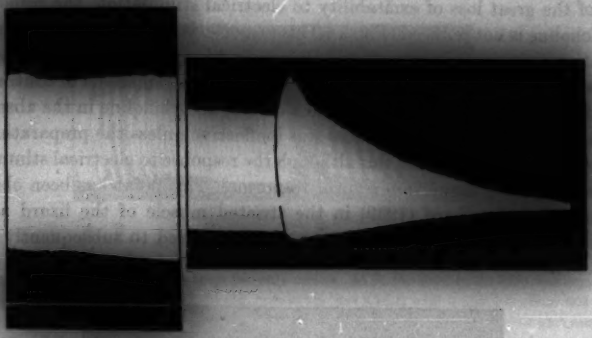

Fig. 1. The response to potassium (24 mg. in 50 c.c. bath) of (*A*) normal diaphragm, and (*B*) the denervated other half diaphragm of the same animal. Time in sec.

Fig. 2. The response of *denervated* diaphragm to acetylcholine (100 μ. Ach) at *A* before and after a depressant dose of potassium (24 mg., at *B*) which renders it inexcitable to electrical stimulation. The response to *A* was abolished by 100 μ. D-tubocurarine, added at *C*. Time in sec.

If the muscle is left in the high-potassium-concentration bath fluid its contractions continue to decline until it ceases to respond, even to stimuli previously maximal or which earlier produced a tetanus. This is due not to failure of contractility but to a very great change in excitability, for by greatly increasing the stimulus by reducing the amount of fluid in the bath, the muscle can be made to contract as before. The reduction cannot be due to the addition of an electrolyte. Calculations based on the response of a curarized muscle partially and completely immersed in fluid indicate that a voltage of 50 times the strength previously required may fail to excite when excitability

becomes depressed. If more potassium is added, the muscle eventually becomes inexcitable to all strengths of stimuli.

If a denervated preparation is used similar changes are produced, but in spite of the great loss of excitability to electrical stimulation, the response to acetylcholine is not reduced (Fig. 2). This response is a sluggish contraction but relaxation may occur in two stages (Dale & Gaddum, 1930), one rapid and the other slow and often incomplete, leaving a contraction remainder or contracture. It is of interest to note that after such a dose of acetylcholine in the absence of excess potassium a subsequent dose was ineffective unless the preparation was washed in fresh solution and this although the response to electrical stimulation after a temporary depression returns to normal. This point has been observed by Buchtal & Lindhard (1939) in the striated muscle of the lizard and by Rosenblueth & Luco (1937) in the cat, and is referred to subsequently in the discussion.

Fig. 3. Effect of adding glucose at *G* in restoring the nerve response to a preparation which has been depressed by lack of glucose and excess potassium. The nerve and muscle were stimulated in alternate groups at *M* and *N*. In the early part of the record the onset of the block is seen. Time in sec.

Effect of neuromuscular block. Production of neuromuscular block by either D-tubocurarine or by lack of glucose (Hajdu & McDowall, 1949) increases the depressant effect of raised potassium concentration in a normally innervated preparation. If the neuromuscular transmission has been completely blocked by glucose lack, the addition of glucose brings about recovery although the extra potassium is still present (Fig. 3). The response to acetylcholine, however, is not sensitized by lack of glucose, e.g. acetylcholine in doses which cause contraction of a denervated preparation is still without effect on the innervated preparation.

When the neuromuscular transmission is blocked by the addition of D-tubocurarine to the bath, there is a similar increase in the sensitivity of the muscle to potassium. Much the most striking effects are, however, produced by adding the potassium first in doses which are known to depress denervated

muscle but which have no effect on the innervated preparation. Now if curare is added, not only is the response to nerve stimulation with a given stimulus abolished but all response of the muscle to a stimulus originally maximal is lost (Fig. 4).

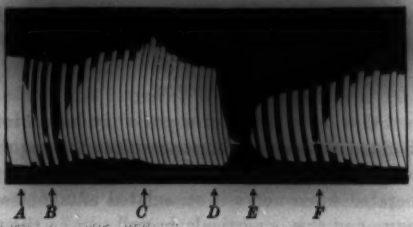

Fig. 4. At *A*, a test dose of D-tubocurarine was given, the muscle and nerve being stimulated alternately in groups. The nerve response only ceases. At *B*, the fluid in the bath was replaced by normal Krebs solution. At *C*, 24 mg. potassium was added with negligible effect but now the addition of D-tubocurarine at *D* causes a cessation of response of both nerve and muscle. At *E*, the additional potassium only was removed by changing the fluid and the muscle recovered; and at *F*, the D-tubocurarine was removed and the nerve recovered. Time in sec.

Fig. 5. Effect of temperature on the response to nerve stimulation. The depression produced by extra potassium (added at *A*) is relieved by the reduction of the temperature at the arrow from 37° to 32° C. Time in sec.

The effect of temperature. The response of the rat diaphragm to amounts of potassium around the concentration used above is extremely sensitive to change of temperature. Thus an increased concentration of potassium at 37° C. produces a typical augmentation followed by a depression, but the depression is recovered from if the temperature is reduced to 32° C. (Fig. 5). Direct and indirect stimulation are affected equally. Similar effects are produced on a denervated preparation. The relation of the action of potassium to temperature

has already been referred to by Quilliam & Taylor (1947) in connexion with its action on curare. Even at room temperature similar results are obtained but an increased amount (5 or 10 mg. in the bath) of potassium may be necessary. At this lower temperature it is easy to demonstrate that large doses of potassium depress neuromuscular transmission before muscle is affected, though stimuli of frequency high enough to produce a tetanus may still be effective.

DISCUSSION

It has been shown that denervation or a procedure which interferes with the acetylcholine liberated at nerve endings sensitizes the rat diaphragm to the depressant action of potassium. From this we may conclude that the normal acetylcholine liberated when nerve is stimulated antagonizes the depressant action of potassium, and this is particularly well seen in the ability of higher-frequency stimuli to pass a block sufficient to stop stimuli of low frequency. How it may do so is a matter of some interest. We have already seen that potassium in small doses stimulates muscle especially at temperatures below normal, while larger doses, on the other hand, depress, commonly after an augmentation. Since it is known that potassium antagonizes curare, it may be suggested that the augmentary phase of potassium action is at least in part through an acetylcholine mechanism and this is supported by the finding that eserine augments this action (Wilson & Wright, 1936). This, however, cannot be the whole explanation, for it has been pointed out by Magladery & Solandt (1942) that potassium will actually stimulate skeletal muscle and especially when it is denervated. It may be that potassium stimulates the synthesis or liberation of acetylcholine, for it has been shown by Feldberg (1944) that potassium increases such synthesis in brain slices, and this view is supported by the observation that the depression is less at lower temperatures, which also increase the synthesis of acetylcholine (Feldberg, 1944).

It is more difficult to explain why the response to acetylcholine in the denervated muscle during potassium depression of excitability to electrical stimulation remains unaltered. This observation, taken together with the observation that a second dose of acetylcholine may be ineffective while the response to electrical stimulation returns to normal, supports the suggestion of Buchtal & Lindhard (1939) that there may be two modes of access to the end-plate.

The depressant action of potassium, we have seen, is enhanced by denervation, by neuromuscular block and by eserine, depending on the dose. With these considerations it is now possible to explain the apparent depressant action of curare on muscle after a stimulating dose of potassium, thus. In normal muscle if the dose of potassium is suitably chosen, the augmentary effects through acetylcholine and the depressant effects on muscle nearly balance each other. When, therefore, the augmentary effect through acetyl-

choline is removed by curare, denervation or other means, the depressant effect on muscle is unopposed.

These findings open up a still more important question as to whether the normal activity of acetylcholine is the antagonism of the potassium of muscle as has been suggested by Verzar (1943). This is being made the subject of a subsequent investigation.

SUMMARY

1. Extra potassium first increases and, in large doses, then depresses the excitability of muscle at body temperature in Krebs's solution.

2. At room temperature neuromuscular transmission is depressed by extra potassium, and eventually a neuromuscular block is produced which, like that produced by curare, can be antagonized by more rapid stimulation.

3. The depression of the muscle is enhanced by denervation and it is suggested that the acetylcholine liberated normally antagonizes potassium depression.

We are indebted to Dr J. E. Hall for confirming the observations on the effect of temperature, and Fig. 5 is from his work.

REFERENCES

Buchtal, F. & Lindhard, J. (1939). *J. Physiol.* **95**, 59*P*.
Dale, H. H. & Gaddum, J. H. (1930). *J. Physiol.* **70**, 109.
Feldberg, W. (1944). *J. Physiol.* **103**, 367.
Gasser, H. S. & Dale, H. H. (1926). *J. Pharmacol.* **28**, 287.
Hajdu, S. & McDowall, R. J. S. (1949). *J. Physiol.* **108**, 502.
Magladery, J. W. & Solandt, D. W. (1942). *J. Neurophysiol.* **5**, 356.
Quilliam, J. P. & Taylor, D. B. (1947). *Nature, Lond.*, **160**, 603.
Rosenblueth, A. & Luco, J. V. (1937). *Amer. J. Physiol.* **120**, 781.
Verzar, F. (1943). *Theorie und Muskelkontraction.* Basel: Schwabe.
Wilson, A. T. & Wright, S. (1936). *Quart J. exp. Physiol.* **26**, 128.

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THE TRANSFER OF L-ASCORBIC ACID AND DEHYDRO-L-ASCORBIC ACID INTO THE AQUEOUS HUMOUR OF THE RABBIT AND CAT

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The concentration of ascorbic acid in the aqueous humour of the rabbit is generally some 20–30 times that in the plasma (Bellows, 1944). On raising the concentration of ascorbic acid in the plasma, a rise in the concentration of ascorbic acid in the aqueous humour occurs, even though the raised plasma level may be considerably below that of the aqueous humour (Tatsumi, Nagao, Okamura & Gamo, 1935; Kinsey, 1947). In the cat's aqueous humour there is no such marked accumulation of ascorbic acid, although again, under steady state conditions, the concentration of ascorbic acid in the aqueous humour is greater than that in the plasma. In the present paper, experiments on the dynamic aspects of the transfer from blood to aqueous humour of ascorbic acid and of its reversibly oxidized form, dehydro-ascorbic acid, will be described. By making a comprehensive study of the dynamics of penetration of these two substances, it was hoped to throw light on the mechanism by which active transport of ascorbic acid across a biological barrier occurs.

METHODS

General. Raised plasma concentrations of the compounds were maintained by periodic intravenous injections. Whilst it was found necessary to anaesthetize the cats lightly with Nembutal, experiments on rabbits were carried out without a general anaesthetic. Removal of the lens from one eye of the rabbit was made using an extra capsular technique. At least 4 months were allowed for recovery to be complete, during which time slit-lamp examination was made to detect any abnormality in the operated eye.

Chemical. Pure dehydro-ascorbic acid (m.p. 224° C.) was prepared by the oxidation of L-ascorbic acid with iodine in methyl alcohol (Kenyon & Munro, 1948). For the kinetic studies, ascorbic acid was dissolved in water, neutralized to pH 7·2 and made up to an isotonic concentration immediately before the experiment. The solution of dehydro-ascorbic acid was made similarly, but no neutralization was necessary as the compound itself is neutral and only slowly hydrolyses to diketogulonic acid. Ascorbic acid was determined by a modification of the electrometric titration procedure of Mindlin & Butler (1938) in which a solution of 2>6-dichlorophenol-indophenol is used

as oxidant. This procedure enabled estimations to be completed within 10 min. of the withdrawal of the fluid sample, the titration being completed in a standard time of 90 sec. The method of Roe & Kuether (1943) was used for the estimation of dehydro-ascorbic acid.

RESULTS

The penetration of ascorbic acid and dehydro-ascorbic acid into the rabbit's aqueous humour

Table 1 records the increase in concentration of ascorbic acid in the aqueous humour following periodic intravenous injection of freshly prepared isotonic sodium ascorbate solution (pH 7·2). The first aqueous humour, withdrawn

TABLE 1. Change in concentration of ascorbic acid in the aqueous humour following a period of raised concentration of ascorbic acid in the plasma

Plasma		Aq. I	Aq. II	Time
AA	DHA	AA	AA	(min.)
14–18	—	17·0	27·0	64
6–10	—	25·8	37·8	40
2–4	0·4	25·2	35·6	67
2–4	0·2–0·5	26·6	32·4	60
2–3	0·5	17·9	34·7	60
13–17	0·5–1·0	17·5	31·0	60

Results in all tables are expressed in mg./100 ml. of aqueous solution. AA = ascorbic acid; DHA = dehydro-ascorbic acid; Aq. I and Aq. II = aqueous humour before and after raising plasma level of ascorbic acid.

before initiation of the experiment, was used as control, the second being withdrawn at the end of the experimental period. Unconscious and conscious animals were found to react similarly. The results support the findings of Tatsumi *et al.* (1935) and Kinsey (1947) that ascorbic acid passes into the aqueous humour against a concentration gradient. The magnitude and rate of increase of the concentration in the aqueous humour were found to be variable. The maximal accumulation of ascorbic acid in the aqueous humour, against a concentration gradient, was 40–50 mg./100 ml. Above this ceiling level it was found that ascorbic acid can still pass in by a diffusion process, i.e. down a concentration gradient.

Estimations revealed that there was no increase in the concentration of dehydro-ascorbic acid in the aqueous humour, although a slight increase in plasma concentration was always recorded.

The effects of raising the concentration of dehydro-ascorbic acid in the plasma are recorded in Table 2. No significant increase in the concentration of dehydro-ascorbic acid in the aqueous humour occurred in any experiment. In contrast, the concentration of ascorbic acid in the aqueous humour rose considerably.

Thus, a rise in the concentration of dehydro-ascorbic acid in the plasma leads to an accumulation in the aqueous humour of its reduced product, ascorbic acid. The maximal concentration of ascorbic acid obtained in the aqueous humour

under these experimental conditions varied between 50 and 70 mg./100 ml. of aqueous solution (see Tables 2 and 3), a level higher than that found in the studies with ascorbic acid.

TABLE 2. Change of concentration of ascorbic and dehydro-ascorbic acids in the aqueous humour following a period of raised concentration of dehydro-ascorbic acid in the plasma

Plasma		Aq. I		Aq. II		Time
DHA	AA	DHA	AA	DHA	AA	(min.)
7–11	1–2	0·2	15·1	0·4	48·6	60
10–15	1–2	0·1	11·0	0·2	35·6	60
20–25	2–4	0	8·9	0	29·0	20
13–17	0·5–1	0·2	12·4	0·3	31·6	45
2–4	0·5–1	0	15·0	0	25·2	45
2–3	0·5	0	21·0	0	46·0	55

For symbols see Table 1.

Table 3 shows the effects of raising the plasma concentration of dehydro-ascorbic acid when the concentration of ascorbic acid had been raised to its ceiling value by intravenous injection of this substance. The results show that dehydro-ascorbic acid passes into the aqueous humour under these conditions.

TABLE 3. Penetration of dehydro-ascorbic acid following an attainment of the ceiling level of ascorbic acid in the aqueous humour

Plasma concentration	Aq. I		Aq. II		Time
DHA	DHA	AA	DHA	AA	(min.)
40–50	0·5	46·5	11·0	43·5	60
30–35	0·4	47·0	3·5	43·0	60
5–10	0·3	36·5	0·8	44·0	50
30–35	0·8	50·0	8·0	69·0	45

For symbols see Table 1.

It appears probable, from these experimental results, that the same mechanism operates to bring about accumulation of ascorbic acid in the aqueous humour, whether this is brought about by a raised plasma concentration of ascorbic acid or of the reversibly oxidized form, dehydro-ascorbic acid. Further, this accumulation may be dependent on a reduction of dehydro-ascorbic acid by the tissues of the eye.

The influence of the lens on the ascorbic acid concentration in the aqueous humour of the rabbit

The lenses of many species have been found to contain appreciable amounts of ascorbic acid; this fact, together with the observation that the amounts of ascorbic acid in the aqueous humour and lens decrease with the development of cataract, gave grounds for the theory that the accumulation of ascorbic acid in the aqueous humour was the result of a synthesis by the lens. Huymans & Fischer (1941) have shown that an increase in ascorbic acid occurs in the excised lens at the expense of loss of glucose. Other workers have been

unable to confirm this conclusion; Bakker (1936) described a technique which kept the lens transparent for 21 days, during which time no synthesis of ascorbic acid was observed.

Initial experiments showed that the removal of the lens causes a decrease in the concentration of ascorbic acid in the aqueous humour; removal of the lens affects the concentration of dehydro-ascorbic acid in the sense that it tends to rise. Thus, the concentration of ascorbic acid in the aqueous humour of the normal eye was $31{\cdot}2 \pm 1{\cdot}1$ (5) and the dehydro-ascorbic acid concentration $0{\cdot}2 \pm 0{\cdot}1$ (5), whilst in the aphakic fluid the ascorbic acid concentration was $9{\cdot}5 \pm 2{\cdot}5$ (5) and the dehydro-ascorbic concentration $0{\cdot}6 \pm 0{\cdot}2$ (5). These results are in keeping with the findings of Muller, Buschke, Gurewitsch & Bruhl (1934) and Bietti (1935). The possibility of achieving, in the aphakic eye, the same ceiling concentration of ascorbic acid as that in the normal aqueous humour was investigated; it was shown that, whether this is attempted by raising the concentration of ascorbic acid or of dehydro-ascorbic acid in the plasma, the maximal value is always less than half that found in the normal eye (Table 4).

TABLE 4. The effect of the lens on the maximum concentration of ascorbic acid obtainable in the aqueous humour of the aphakic eye

Plasma concentration		Aphakic aqueous humour	Normal aqueous humour	Time
AA	DHA	AA	AA	(min.)
15–20	—	29·0	58·0	150
15–20	—	22·7	58·5	150
10–15	—	27·8	61·5	150
10–15	—	9·6	40·5	90
—	10–15	17·8	39·0	60
—	20–25	17·8	43·7	80

For symbols see Table 1.

In the last two experiments in which the concentration of dehydro-ascorbic acid in the plasma had been raised, 5·6 and 3·8 mg./100 ml. of dehydro-ascorbic acid were found in the aphakic fluid, contrasting with 2·3 and 0·4 mg./100 ml. in the normal eyes. Thus, removal of the lens leads to a decrease in capacity to accumulate ascorbic acid and an increase in capacity to accumulate dehydro-ascorbic acid. These results are in keeping with those recorded in Table 3, where it will be seen, that, following saturation of the secretory process, dehydro-ascorbic acid accumulates in the eye.

The penetration of ascorbic acid and dehydro-ascorbic acid into the cat's aqueous humour

The concentration of ascorbic acid in the aqueous humour of the cat varied between 0·5 and 2·0 mg./100 ml., and the normal plasma values varied between 0·5 and 1·0 mg./100 ml. Thus, although the concentration of ascorbic acid in the cat's aqueous humour is very much less than in that of the rabbit (Bellows, 1944), there is some evidence of accumulation on a smaller scale. Samples of

aqueous humour withdrawn from a pair of eyes showed no significant difference in concentration. Certain characteristics of the transfer of these two compounds across the blood aqueous barrier were made apparent in the initial kinetic studies. First, both ascorbic and dehydro-ascorbic acids pass across the barrier into the aqueous humour. Secondly, there is no marked inter-corrosion of the two substances during the transfer. Finally, they pass the barrier at a similar speed. Thus, if this rate of penetration is expressed as the parameter K ($\times 100$) (Davson & Quilliam, 1940), we have:

K ($\times 100$) for ascorbic acid $= 4{\cdot}1 \pm 0{\cdot}6$ (S.E. of mean of twelve observations);
K ($\times 100$) for dehydro-ascorbic acid $= 5{\cdot}8 \pm 0{\cdot}4$ (6).

DISCUSSION

The results obtained with ascorbic acid in the rabbit are in general agreement with those reported by Kinsey (1947); the extension of the work to the study of dehydro-ascorbic acid has revealed that injection of this compound into the blood stream causes an accumulation of ascorbic acid in the aqueous humour even larger than that produced by ascorbic acid itself. The studies with aphakic eyes show that the absence of the lens diminishes the accumulation of ascorbic acid in the aqueous humour and increases the accumulation of dehydro-ascorbic acid. The presence of an active secretory mechanism would account for the large difference observed between plasma and aqueous humour concentrations of ascorbic acid, but the observations also seem to suggest that much if not all the ascorbic acid is transferred into the aqueous humour as dehydro-ascorbic acid, which is reduced to ascorbic acid by intra-ocular mechanisms, one site of reduction being the lens. However, a contribution to the ascorbic acid content of the aqueous humour by a synthetic process occurring in the lens, as claimed by Huymans & Fischer (1941), cannot be ruled out.

In the cat, the studies indicate that there is no marked accumulation of ascorbic acid in the aqueous humour, whether this is attempted by raising the concentration of ascorbic or dehydro-ascorbic acids in the blood; the kinetic studies suggest, moreover, that both compounds pass across the blood-aqueous barrier by simple diffusion. The only indication of an active process for transfer of ascorbic acid in the cat is in the observation that the concentration of ascorbic acid in the aqueous humour is higher than that found in the plasma. Under the experimental conditions reported here, no further evidence of a secretory process analogous to that found in the rabbit could be observed. It would be unwise to conclude that this secretory process is absent in the cat, for the dynamics of intra-ocular fluid formation may vary between the two species; this view is supported by the fact that the ratio of volume of aqueous humour to that of the lens is much greater in the cat than in the rabbit.

SUMMARY

1. In the rabbit, raising the concentration of dehydro-ascorbic acid in the blood leads to a marked increase in the concentration of ascorbic acid in the aqueous humour; however, the concentration of dehydro-ascorbic acid in the aqueous humour during this time remains unchanged.

2. A ceiling level of ascorbic acid in the aqueous humour (*c.* 50 mg./100 ml.) results from a raised concentration in the plasma of either ascorbic or dehydro-ascorbic acid.

3. In the rabbit with one aphakic eye, kinetic studies show that the ceiling level of ascorbic acid attained in the aphakic eye is always much less than in the normal eye, although the dehydro-ascorbic acid concentration is always higher than in the normal eye.

4. It is suggested that accumulation of ascorbic acid in the aqueous humour of the rabbit involves the reduction of dehydro-ascorbic acid by the lens and other tissues of the eye.

5. In the cat, the accumulative mechanism is far less efficient, so that on raising the concentration of either acid in the blood, penetration is largely determined by the concentration gradient.

I wish to thank Dr H. Davson for his interest and help given during this work; and the Medical Research Council for defraying the cost of this research.

REFERENCES

Bakker, A. (1936). *Arch. Ophthal., N.Y.*, **136**, 166.
Bellows, J. G. (1944). *Cataract and Anomalies of the Lens*, 1st ed. p. 230. London: Kimpton.
Bietti, G. (1935). *Boll. Oculist.* **14**, 3.
Davson, H. & Quilliam, J. P. (1940). *J. Physiol.* **98**, 141.
Huymans & Fischer (1941). *Ophthalmologica*, **102**, 275.
Kenyon, J. & Munro, N. (1948). *J. Chem. Soc.* p. 158.
Kinsey, V. E. (1947). *Amer. J. Ophthal.* **30**, 1262.
Mindlin, R. L. & Butler, A. M. (1938). *J. biol. Chem.* **122**, 673.
Muller, K. H., Buschke, W., Gurewitsch, A. & Bruhl, F. (1934). *Klin. Wschr.* **13**, 20. (Cited in Bellows, J. G. (1944). *Cataract and Anomalies of the Lens*, 1st ed. p. 232. London: Kimpton.)
Roe, J. & Kuether, C. (1943). *J. biol. Chem.* **147**, 399.
Tatsumi, M., Nagao, Y., Okamura, K. & Gamo, J. (1935). *Klin. Wschr.* **14**, 1007.

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THE INTRINSIC INDEPENDENCE OF BLOOD FLOW THROUGH CORTICAL AND JUXTA-MEDULLARY GLOMERULI

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Trueta, Barclay, Daniel, Franklin & Prichard (1947) reported changes in the distribution of dyes, ink and radio-opaque material in the outer and inner portions of the cortex and subcortical medulla of the kidneys of animals after various procedures. In most instances they found a decreased injection of the cortex, particularly of the peripheral two-thirds, apparently the result of vasoconstriction, while there was progressively less change towards the central portions. These observations were interpreted as indicating that 'the blood passing through the kidney is diverted from the cortex to the medulla'. A complementary type of diversion due to activity of medullary vessels was also postulated: '...the intrarenal blood flow can in some cases be diverted from the cortex...as a result of a dilation of the vessels associated with the juxtamedullary glomeruli....Apparent diversion of...blood flow from medulla to cortex [with] the circulation through the cortex...increased while the circulation through the medulla was decreased' was also described. The direction of diversion in this case was opposite to the more usual one.

The word 'diversion' is used by these authors to indicate that the proportion of renal blood flowing through cortex and subcortical medulla is changed, a statement which *need* imply no intrinsic dependence of blood flow rate through one of these regions on the rate of flow through the other. However, Trueta *et al.* (1947) and other investigators seem to imply such an interdependence of blood flow between these two regions and to attach considerable physiological and pathological significance to its existence. If there were no such interdependence, or if it were quantitatively insignificant, there would be no need constantly to refer to the medullary by-pass whenever the effects of cortical ischemia are in question. As, for instance: 'It seems possible, therefore, that excessive diversion of the intrarenal blood flow from the cortex through the medullary by-pass may be an initiating factor in the production of the pressor substance and thus in the development of human essential hyper-

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tension....Normally the medullary pathway appears in most instances to serve passively as a by-pass for the diverted cortical blood flow.' (As though cortical ischemia requires the presence of this by-pass.) '...the blood which was denied access to the cortical pathway was able nevertheless to make an intrarenal circuit through the medullary by-pass.'

An analysis has therefore been made of the relationship between rate of blood flow through the juxtamedullary glomeruli and through cortical glomeruli when either the cortical vessels alone or the juxtamedullary vessels alone are presumed to change in calibre and under certain other haemodynamic conditions designed to discover the maximal interdependence intrinsic in the vascular relationship between these two regions. Some of the haemodynamic conditions referred to are these:

(1) Approximately correct results can be obtained by treating the blood in the kidney as a viscous rather than pseudoplastic fluid (Lamport, 1949) which means: pressure drop is proportional to blood flow rate through any portion of the renal circulation as long as there is no change in the vessel calibre (the hindrance):

$$\text{Pressure drop} = \text{Flow rate} \times \text{Hindrance}.$$

(2) Aortic blood pressure is unchanged and a single pressure value can effectively replace pulsating arterial pressure. 100 mm. Hg is taken for this value, but any other can be substituted.

(3) The vascular connexions between the two regions under study are approximately represented by Fig. 1 in which i refers to blood flow rate, h to the hindrance, and the arrows indicate pressure difference. The routes represented by dashed lines correspond to the pathways of glomerular filtrate. They are neglected in this analysis with little consequent error because the reduction in the volume of the blood due to passage of the filtrate into the tubular system is offset in large part by the resultant increase in the viscosity of the blood (Lamport, 1941).

Case I. Cortical vasoconstriction

When the mechanism studied by Trueta *et al.* is evoked, $i_c \rightarrow i'_c$ and $i_j \rightarrow i'_j$ but unchanged are h_j, h_a and h_v, referring respectively to the hindrances of juxtamedullary vessels, to those arterial vessels shared in common by both regions of the kidney, and to those venous vessels similarly shared. The following algebraic statements embody their observations under the stated haemodynamic conditions; their meaning is made clearer by reference to Fig. 1:

$$P = ih_a, \quad \mathscr{P} = ih_v,$$

$$100 = \mathscr{P} + i_j h_j + P = i_j h_j + i(h_a + h_v).$$

Since $i = i_j + i_c$,

$$100 = i_j(h_j + h_a + h_v) + i_c(h_a + h_v),$$

and

$$i_j = \frac{100 - i_c(h_a + h_v)}{h_j + h_a + h_v}.$$

Similarly, when $i_c \to i'_c$,

$$i''_j = \frac{100 - i''_c(h_a + h_v)}{h_j + h_a + h_v}.$$

Let $\Delta i_j = i''_j - i_j$, the increase in juxtamedullary blood flow rate resulting from cortical vasoconstriction:

$$\frac{\Delta i_j}{i_j} = \frac{(i_c - i''_c)\,(h_a + h_v)}{100 - i_c(h_a + h_v)}.$$

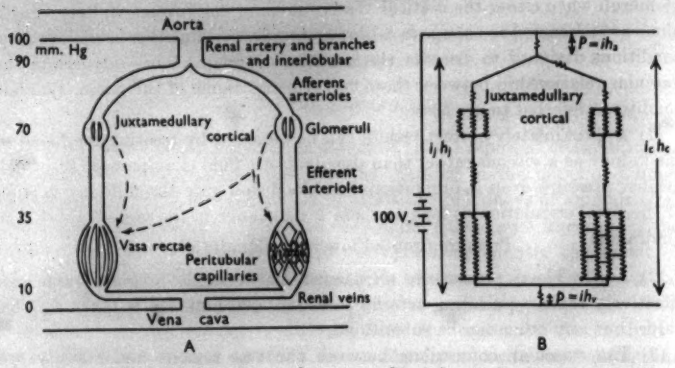

Fig. 1. See text.

Since $h_a = P/i$ and $h_v = \mathscr{P}/i$,

$$h_a + h_v = \frac{P + \mathscr{P}}{i}.$$

Since $h_a + h_v$ are constants, we can evaluate them under any convenient circumstance. We choose the control condition, where $P = P_0$, $\mathscr{P} = \mathscr{P}_0$ and $i = i_0$:

$$h_a + h_v = \frac{P_0 + \mathscr{P}_0}{i_0},$$

$$\frac{\Delta i_j}{i_j} = \frac{P_0 + \mathscr{P}_0}{100 - (P_0 + \mathscr{P}_0)\, i_c/i_0} \left(\frac{i_c}{i_0} - \frac{i''_c}{i_0} \right).$$

(4) We now add another haemodynamic condition. Since we are seeking the maximal interdependence between i''_j and i''_c as a result of change in i_c to i'_c, we shall choose those values of i_c/i_0 and i''_c/i_0 which will maximize $\Delta i_j/i_j$. The appropriate extreme limiting conditions for juxtamedullary diversion due to cortical vasoconstriction are

$$i_c/i_0 \to 1 \quad \text{and} \quad i''_c/i_0 \to 0.$$

As a result,

$$\frac{\Delta i_j}{i_j} < \frac{P_0 + \mathscr{P}_0}{100 - (P_0 + \mathscr{P}_0)}.$$

P_0 is the pressure drop in the renal artery and its branches up to the arterial point where the two regions no longer share the same vessels (Fig. 1*B*). $\mathscr{P}_0$ is the pressure drop in the renal veins and its tributaries up to the point where blood from the cortical and juxtamedullary regions becomes confluent. It is unlikely that $P_0+\mathscr{P}_0$ is greater than 10 mm. Hg. Thus

$$\frac{\Delta i_j}{i_j}<\frac{1}{9}, \quad \text{or } 11\%.$$

Case II. Juxtamedullary vasoconstriction

Correspondingly, the same argument applies to the effects of juxtamedullary vasoconstriction on cortical flow, another type of shunting. We have

$$\frac{\Delta i_c}{i_c}=\frac{P_0+\mathscr{P}_0}{100-(P_0+\mathscr{P}_0)\,i_j/i_0}\left(\frac{i_j}{i_0}-\frac{i''_j}{i_0}\right).$$

Our limiting conditions to favour the shunting hypothesis are now

$$i_j/i_0\to 1 \quad \text{and} \quad i''_j/i_0\to 0.$$

They easily yield the result

$$\frac{\Delta i_c}{i_c}<\frac{P_0+\mathscr{P}_0}{100} \quad \text{and} \quad \frac{\Delta i_c}{i_c}<10\%.$$

Case III. Juxtamedullary vasodilation

For the sake of completeness we consider the remaining possibilities. What is the effect on cortical flow if juxtamedullary vasodilation is induced, an unusual shunting mechanism referred to by Trueta and his associates? Returning to the expression immediately above for $\frac{\Delta i_c}{i_c}$, we choose as limiting conditions $i_j/i_0\to 0$ and $i''_j/i_0\to 1$. The latter condition requires some explanation. It must be recalled that of all the renal glomeruli the juxtamedullary glomeruli are only a small minority, about 15% in the rabbit (Heggie, 1947). The condition then requires, taking the rabbit as our example, that vasodilation increase juxtamedullary blood flow about seven times more than its proportionate share of normal renal blood flow. Pappenheimer & Maes (1942) found that blocking the vascular nerve supply in the isolated hindlimb of the dog decreased its hindrance by about 25%, so that the sevenfold factor here offered seems a generous estimate for obtaining a favourable test of this variation of the shunt hypothesis; active vasodilation is not markedly superior to nerve block.

The result of inserting these conditions into our expression is

$$\frac{\Delta i_c}{i_c}>-10\%.^*$$

Or, in words, the maximal possible decrease in cortical flow that can result from juxtamedullary shunting is less than 10%.

* Δi_c and Δi_j are defined as increases in flow rate. A negative value indicates a decrease in flow.

Case IV. Cortical vasodilation

Diversion of blood from the juxtamedullary glomeruli by cortical vasodilation remains to be treated. Our original expression for $\Delta i_j/i_j$ in case I, before the substitution for h_a+h_v, is most convenient. To favour strongly the shunt hypothesis, we set $i_c=0$, the most favourable condition. We then have

$$\frac{\Delta i_j}{i_j} = -\frac{i'_c(h_a+h_v)}{100}.$$

Since we have markedly altered total renal blood flow by our condition that roughly 85% of it is nil, because $i_c=0$, we evaluate the constant (h_a+h_v) under *normal* conditions, when the pressure drop along the two hindrances is estimated at 10 mm. Hg and the flow through them we call i_{00}. Thus $h_a+h_v=10/i_{00}$.

For our final condition we let $i'_c \to 1{\cdot}25 i_{00}$. Assuming proportional flow of blood to all glomeruli irrespective of their location under normal conditions, and accepting the 85% figure for the fraction of all glomeruli which are cortical, our last condition amounts to considering the augmentation of cortical blood flow above normal, as a result of active vasodilation, to be 1·25/0·85 or 47%. Such a figure again seems a reasonably generous choice for testing the shunt hypothesis; it leads to

$$\frac{\Delta i_j}{i_j} > -13\%.$$

The reduction in juxtamedullary blood flow resulting from cortical vessels dilating maximally after being shut down completely is less than 13%.

SUMMARY AND CONCLUSION

It must be concluded that even under the most extreme conditions there is little dependence of cortical and juxtamedullary blood flow on one another directly, in an intrinsic manner, as a result of their haemodynamic connexions. Dependence of juxtamedullary and cortical blood flow rates on one another, if significant, must therefore be largely of extrinsic origin—the result of a difference in response of their vessels to the drugs, hormones, neural stimulation, and other agencies studied by Trueta and his associates.

REFERENCES

Heggie, J. F. (1947). *Lancet*, **1**, 385.

Lamport, H. (1941). *J. clin. Invest.* **20**, 535.

Lamport, H. (1949). 'Hemodynamics.' *Textbook of Physiology.* Ed. Fulton, J. F. 16th ed. Philadelphia: Saunders.

Pappenheimer, J. R. & Maes, J. P. (1942). *Amer. J. Physiol.* **137**, 187.

Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J. & Prichard, M. M. L. (1947). *Studies of the Renal Circulation.* Springfield, Illinois: Thomas.

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AFFERENT NERVES FROM THE HEART REGION

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In recent years considerable work has been done to investigate the behaviour of the different pressoreceptors found in the walls of blood vessels. Endings of this sort were suggested by Bainbridge (1915) to be the receptors responsible for the reflex which bears his name. Sassa & Miyazaki (1920) extended the original observations, but later workers cast doubt on the regularity with which the effect could be observed. Tiitso (1937) claimed that the cardioacceleration following a rise of venous pressure was a local reaction of the heart; and Ballin & Katz (1941) suggested that pressoreceptors in the great veins and right atrium could not be solely responsible for the Bainbridge reflex. Nonidez (1937, 1941) has shown that subendothelial endings exist in the venous system on both sides of the heart, but their precise function has yet to be elucidated. Amann & Schaefer (1943), Walsh & Whitteridge (1944), Walsh (1947), Whitteridge (1948) and Jarisch & Zotterman (1948) have all recorded impulses derived unmistakably from venous pressoreceptors; but it has so far proved impossible to study the behaviour of these endings accurately, mainly because of the difficulties of accurate recording of effective venous pressure. The first object of these experiments was observation of the activity of these fibres with the chest open and with an improved venous manometer. The types of fibre (other than the venous) which pass to the vagus via its cardiac branches have also been investigated.

METHODS

Satisfactory results have been obtained from fourteen cats, which were in every case deeply anaesthetized with 'Dial', given intraperitoneally half an hour before operation. The chest was opened along the mid-line, under artificial ventilation, and ribs 1–7 inclusive on the right side were ligated close to the vertebral column, using a curved needle from inside the chest. The length of the seventh rib was separated from the eighth, and the first seven ribs were then cut distal to the ligatures. This allowed the upper part of the right chest wall to be reflected as a flap, giving a good exposure of the right vagus and its cardiac branches. A specially shaped glass cannula, 11 cm. long, and 1·2 mm. internal diameter, was inserted by the right external jugular vein, and passed down until its tip could be felt in the right atrium.

After the preparation had been placed in a steam chamber, the cardiac branches of the right vagus were dissected out, using fine cataract knives, and also knives fashioned from small splinters of safety razor blades. Fine dissection was carried out with the aid of a binocular loupe. For recording, a small slip of nerve was cut centrally, laid across the two silver electrodes, and covered

with liquid paraffin to prevent drying. Action potentials, amplified by a conventional five-stage resistance/capacitance coupled amplifier, were displayed on a multichannel system. By this means the electrocardiogram (lead III), right atrial pressure, intratracheal pressure, and a time marker could be simultaneously recorded.

Intratracheal pressure was recorded by a mirror membrane manometer and an optical system, and venous pressure was recorded by a specially designed electrical capacitor manometer (Baxter, 1950). Early trials with optical manometers showed that an adequate sensitivity, combined with a high natural period, could not be obtained. For this reason the venous pressure changes were transmitted by heparin saline in lead tubing to a circular brass diaphragm, $\frac{1}{2}$ in. diameter, and $\frac{11}{1000}$ in. thick—turned from the solid. This diaphragm formed the moving plate of an air capacitor, whose other electrode was a fixed plate, $\frac{5}{1000}$ in. away. The whole capacitor 'head' was designed to be insensitive to changes of temperature and external strains. The capacitance of the head, together with that of the coaxial connecting cable and a variable tuning capacitor, formed part of a tuned circuit, which was driven at approximately 500 kcyc./sec. by a master crystal oscillator. Changes in head capacitance caused by movement of the diaphragm produced changes in resonant frequency; and the phase difference between the resonant frequency and the fixed crystal oscillator frequency was compared with a new type of phase discriminator, which produced a steady output voltage proportional to the phase angle. This output was directly coupled to one deflector plate of the cathode-ray tube display system.

The electrical system, with the cannula and liquid system described, was adequate for the accurate recording of venous pressure. Maximum sensitivity was 6 mm. deflexion on the record for a change of pressure of 10 cm. of water, and the natural period of vibration was 200 cyc./sec. The latter is adequate to reproduce the highest significant frequency components of the venous pulse.

In all cases the frequency of a single unit discharge has been estimated by the reciprocals of the time intervals between impulses, and frequency has been plotted against the mid-points of the time intervals. The resulting histograms have been plotted alongside the electrically recorded pressure changes, to determine the relation between the frequency of discharge of a receptor and the pressure over any given period of time—usually one heart-beat. Since there is no parallax between the cathode-ray tube traces, this method is legitimate.

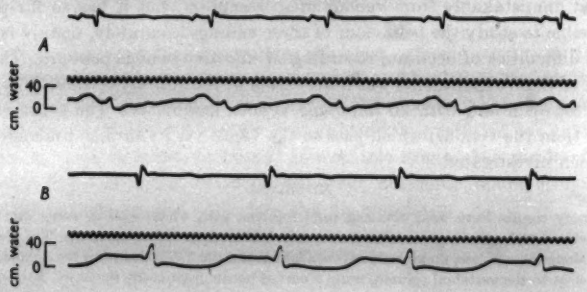

Fig. 1. Two records of right atrial pressure in the cat, made with the electrical capacitor manometer. From above downwards, the e.c.g.; time in 50 cyc./sec.; right atrial pressure. *A*, normal rhythm; *B*, nodal rhythm.

RESULTS

Performance of the venous manometer. A typical record of right atrial pressure, obtained with the liquid system described, is shown in Fig. 1. The chest is open, and artificial ventilation (not shown) is being maintained. Fig. 1*A* shows the

heart in normal rhythm, with the *a*, *c*, and *v* waves clearly visible. Fig. 1*B* shows the same heart 2 min. later, after nodal rhythm had developed. The very large *a* wave, caused by the atrium contracting against closed atrio-ventricular valves, is the most conspicuous feature of this record.

Types of receptor discovered in the cardiac branches of the right vagus

The most conspicuous feature of a typical multifibre record obtained from a large slip of the main cardiac branch of the right vagus is shown in Fig. 2. It consists of the discharge from many venous pressoreceptors in the great veins and right atrium. The discharge can be seen to be approximately related to the venous pressure. In addition to these venous fibres, there are several other types. Since the cardiac branch of the vagus usually receives a small

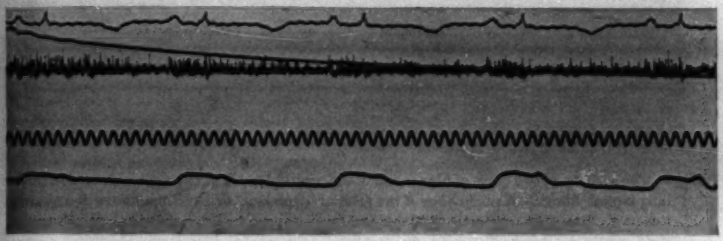

Fig. 2. Multifibre record from a large slip of the cardiac branch of the right vagus, showing conspicuous venous fibre discharge. From above downwards, the e.c.g.; intratracheal pressure; nerve impulses; time in 50 cyc./sec.; right atrial pressure.

contribution from the right lung, typical lung stretch fibres have often been seen, and this has been confirmed by Dawes & Whitteridge (1948). The activity of a single fibre dissected from the nerve examined is shown in Fig. 3*A*. The frequency of discharge is evidently related to the intratracheal pressure. Fig. 3*B* shows a record obtained from a receptor which lies almost certainly in the wall of a vessel of the arterial system, since the position of its discharge in the cardiac cycle is that generally considered typical of an aortic 'depressor' fibre. In Fig. 4 the activity of two fibres identified in another record has been plotted, to show the respective time relations. The fibre whose discharge occurs comparatively late in the cardiac cycle (starting from the *QRS* wave of the e.c.g.) is that from another arterial pressure receptor; but the other fibre, whose discharge is of high frequency and occurs early in the cycle, is clearly of an entirely different type. It may possibly be a 'ventricular' fibre, since its discharge pattern exactly parallels that described by Whitteridge (1948) and presumed to result from the activity of a pressure receptor in the ventricular wall itself.

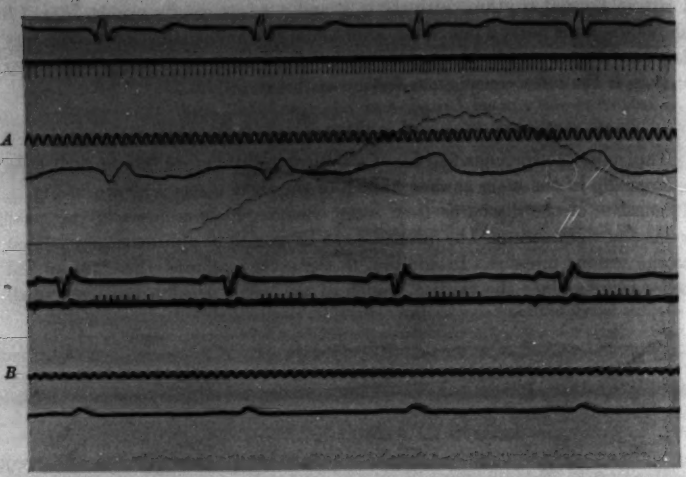

Fig. 3. The activity of two types of fibre found in the cardiac branch of the right vagus. *A*, a single lung stretch fibre; *B*, a single fibre of the arterial 'depressor' type. From above downwards, the e.c.g.; fibre discharge; time in 50 cyc./sec.; venous pressure (*A*, right atrial; *B*, in superior vena cava); intratracheal pressure (in *A* only).

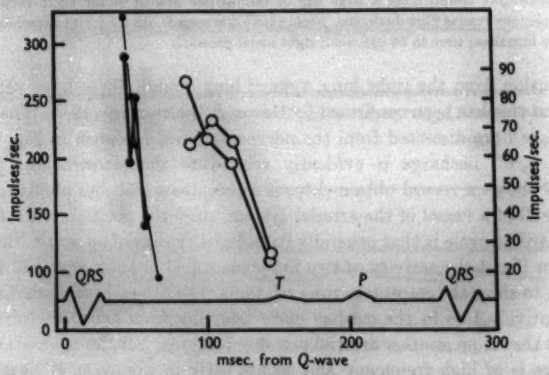

Fig. 4. Record of activity in two fibres recorded simultaneously, in relation to the cardiac cycle. The reciprocal of the interval between successive impulses is plotted as the frequency in impulses per sec. Time is measured from the *Q* wave of the e.c.g., simultaneously recorded. The ordinate scales at the left refer to the fibre discharge shown at the left (black circles); those at the right refer to the other discharge (hollow circles).

The pattern of discharge from pressure receptors in the great veins and right atrium

Fig. 5 shows a record from a small nerve slip containing an 'arterial' fibre of the type already indicated, and a 'venous' fibre. The venous fibre can be seen to discharge twice only, between the *P* and *QRS* waves of the e.c.g., corresponding to the *a* wave of the venous pulse. In this experiment the cannula was in the superior vena cava, and the venous pressure was very low indeed.

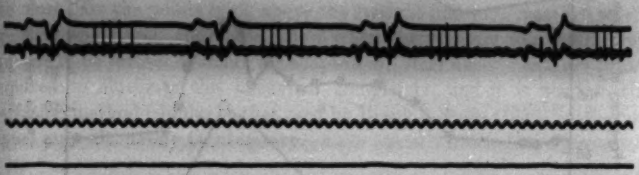

Fig. 5. Record of activity of two different fibres made simultaneously from the same slip of nerve. The pre-systolic discharge (two impulses) comes from a pressoreceptor in the superior vena cava; and the late systolic discharge (five impulses) is from an arterial pressoreceptor. From above downwards, the e.c.g.; nerve impulses; time in 50 cyc./sec.; pressure in the superior vena cava (very low). (The arterial fibre impulses have been retouched to make their position clear when reproduced.)

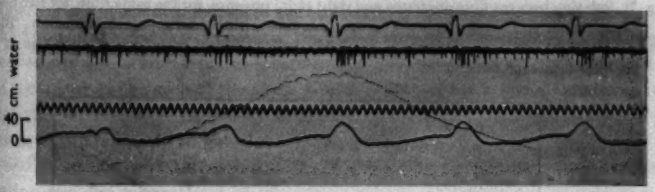

Fig. 6. Record of the activity of a single pressoreceptor in the wall of the right atrium. The heart is in nodal rhythm. From above downwards, the e.c.g.; fibre discharge; time in 50 cyc./sec.; right atrial pressure; intratracheal pressure. (The venous fibre impulses have been retouched to make their position clear when reproduced.)

Even the *a* waves of the venous pulse are scarcely visible, although the manometer sensitivity is the same as that used in Fig. 1 for atrial pressure recording. At higher pressures the pattern of discharge becomes more complex, corresponding to the shape of the venous pulse. Fig. 6 shows the discharge of a pressoreceptor which probably lies in the wall of the right atrium, together with a record of right atrial pressure. (Since the chest was open, this recorded venous pressure represents the actual pressure inside the system, and no correction is necessary for intrapleural pressure changes.) The heart is in nodal rhythm, and the large *a* wave comes after the *QRS* wave of the e.c.g. Fig. 7

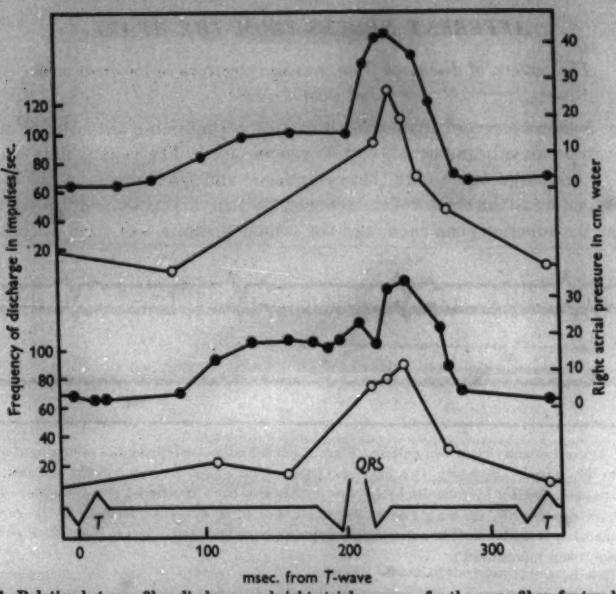

Fig. 7. Relation between fibre discharge and right atrial pressure, for the same fibre, for two heart-beats (upper and lower graphs). The time relations with the e.c.g. are shown below. Black circles: right atrial pressure (scales on right); hollow circles: frequency of discharge of the receptor (scales on left).

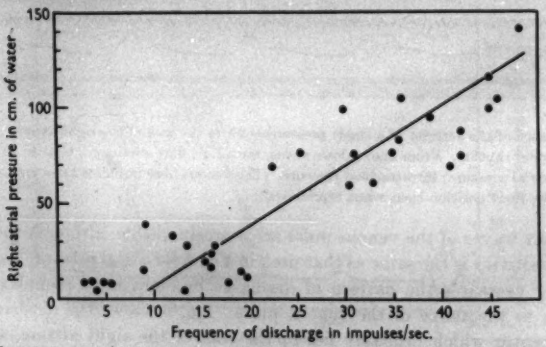

Fig. 8. The relation between pressure and discharge frequency for a pressoreceptor in the right atrium, during six successive heart-beats. The line has been drawn by eye, ignoring the five points at the lower left corner. These represent the intervals between each group of impulses, when the fibre was not discharging, and do not thus indicate any real relation between pressure and frequency of discharge.

shows the discharge frequency of this receptor plotted on a time scale together with pressure, for two complete heart-beats. When the pressure is changing rapidly, the impulse discharge frequency follows the pressure fairly accurately. However, at the beginning of the diastolic *v* wave, Fig. 6 shows that the receptor responds by a single or double discharge, but does not exhibit any further activity while this slight pressure is maintained. There is therefore some evidence for adaptation of the ending. Fig. 8 shows a graph, covering the six heart-beats in Fig. 6, relating frequency of discharge to pressure. It was observed that for the most part the points lying above the straight line were recorded when the pressure was rising, and those below when the pressure was falling—which appears to be further evidence for adaptation. More experiments are evidently required to elucidate this behaviour completely, and it is possible that a perfusion method (similar to that used by Bronk & Stella (1935) for the carotid sinus) may ultimately be necessary.

DISCUSSION

The existence of afferent fibres in the cardiac branches of the right vagus other than those from typical venous pressoreceptors is of some interest. They have not been reported by Jarisch & Zotterman (1948), but these authors comment that Nonidez (1935) discovered typical pressoreceptors in the wall of the descending aorta, giving rise to the 'rami aortici vagi'. These nerves presumably contribute to the cardiac nerves examined; and it seems much more likely that the typical arterial pressoreceptor discharge recorded from fibres in this region represents the activity of receptors in the descending aorta, rather than that of receptors at the arch of the aorta. The presence of these fibres, whatever their derivation, makes it necessary to regard the afferent fibres contained in the main cardiac branch of the right vagus as of more than one type. This is one possible reason why the experiments of Jarisch & Zotterman (1948) on stimulating the cut central end of the nerve were so inconclusive. (Another is, of course, that since the main efferent side of the Bainbridge reflex is represented by a decrease in 'tonic' cardio-inhibitory discharge, cutting off this discharge on one side—by cutting the cardiac branch of the vagus before stimulating it—will greatly reduce the efficiency of the efferent side of the reflex.)

Ballin & Katz (1941) have cast considerable doubt upon the possible functions of the venous pressoreceptors. They were unable to produce a reflex cardio-acceleration in dogs by distending the great veins and right atrium with specially designed balloons; although they were consistently able to produce an increase of heart rate by saline infusion. They therefore suggested that venous pressoreceptors on the right side were not the most important receptors for the Bainbridge reflex. If it is true that the endings adapt to some extent, it might be possible that the stimulus of a sustained distension (such as would be provided by any kind of inflating cannula) is inadequate to produce

a significant increase in discharge of the receptors. However, Megibow, Katz & Feinstein (1943) found that they could consistently produce an increase in rate and depth of breathing, but no change in heart rate, by distending the region where the superior vena cava joins the right atrium. Thus unless other endings (e.g. pain endings) were stimulated, which seems improbable, the distension was probably an adequate stimulus to the venous pressoreceptors. If the conclusions suggested by these experiments are correct, it may be necessary to reconsider completely not only the role of venous pressoreceptors, but the Bainbridge reflex itself. If, however, the stimulus of steady distension is not adequate for the venous pressoreceptors, they may still be concerned in the Bainbridge reflex. It is certainly probable that the normal function of the endings is a response to increased venous return resulting from muscular exercise. Both cardio-acceleration and hyperpnoea would seem to be useful physiological responses.

It is fairly certain, however, that the venous pressoreceptors studied take no part in a 'venopressor reflex' (McDowall, 1924). The type of fibre required by McDowall's hypothesis should have a discharge whose frequency is increased by a fall of pressure. In the course of this investigation, and of many others conducted by different investigators, no fibre of the type required has ever been seen. Recently, Coleridge, Kenney & Neil (1949) have confirmed that the removal of tonic discharge from chemoreceptors (by cutting the vagi) is quite sufficient to produce the conditions of the 'venopressor reflex'; and it does not at present seem necessary to postulate the existence of any new ending.

SUMMARY

1. The afferent activity of the cardiac branches of the right vagus of the cat has been examined by direct electrical recording with the chest open.

2. In addition to typical venous pressoreceptors, lung stretch fibres and arterial pressoreceptors have been found to contribute fibres to these nerves.

3. A linear relation has been found between venous pressure and frequency of discharge in fibres from the right atrium.

4. Some evidence of adaptation of venous pressoreceptor discharge has been found.

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REFERENCES

Amann, A. & Schaefer, H. (1943). *Pflüg. Arch. ges Physiol.* **246**, 757.
Bainbridge, A. (1915). *J. Physiol.* **50**, 65.
Ballin, I. R. & Katz, L. N. (1941). *Amer. J. Physiol.* **135**, 202.
Baxter, I. G. (1950). To be published.
Bronk, D. W. & Stella, G. (1935). *Amer. J. Physiol.* **110**, 708.
Coleridge, J. C. G., Kenney, R. A. & Neil, E. (1949). *J. Physiol.* **110**, 27*P*.

Dawes, G. S. & Whitteridge, D. (1948). Unpublished observations.
Jarisch, A. & Zotterman, Y. (1948). *Acta physiol. Scand.* **16**, 31.
McDowall, R. J. S. (1924). *J. Physiol.* **59**, 41.
Megibow, R. S., Katz, L. N. & Feinstein, M. (1943). *Arch. intern. Med.* **71**, 536.
Nonidez, J. F. (1935). *Amer. J. Anat.* **57**, 259.
Nonidez, J. F. (1937). *Amer. J. Anat.* **61**, 203.
Nonidez, J. F. (1941). *Amer. J. Anat.* **68**, 151.
Sasa, K. & Miyazaki, H. (1920). *J. Physiol.* **54**, 203.
Tiitso, M. (1937). *Pflüg. Arch. ges Physiol.* **238**, 738.
Walsh, E. G. (1947). *J. Physiol.* **106**, 466.
Walsh, E. G. & Whitteridge, D. (1944). *J. Physiol.* **103**, 37*P*.
Whitteridge, D. (1948). *J. Physiol.* **107**, 496.

J. Physiol. (1950) 111, 408–422 547.435.292:612.748

THE ELECTROMOTIVE ACTION OF ACETYLCHOLINE AT THE MOTOR END-PLATE

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Since the work of Dale and his co-workers (Dale, Feldberg & Vogt, 1936; Brown, Dale & Feldberg, 1936; Brown, 1937), evidence has accumulated which shows that acetylcholine (Ach) plays an important part in the transmission of impulses from motor nerve to muscle. According to the now accepted view Ach is liberated on the arrival of an impulse at the nerve terminations and then acts on the muscle fibre so as to give rise to a propagated impulse and contraction. The immediate action of Ach on the muscle fibre is a reduction of the normal resting potential of the fibre membrane (Cowan, 1936) similar to that produced by a stimulating electric current. Kuffler (1943) showed that Ach in low concentrations exerts this depolarizing action only at the end-plate regions of the fibre.

If, in accordance with the classical view, the membrane potential arises from a selective permeability toward different ions, the role of Ach at the motor end-plate could be explained by either of two possible reactions (see Katz, 1942, p. 182). First, the membrane might be rendered permeable to previously non-penetrating ions, which on diffusing across would reduce the resting potential; or, secondly, the Ach ion (Ach^+) itself might move inward across the membrane at a rate sufficiently high to produce a depolarization. The first possibility is of special interest since the investigations of Hodgkin & Katz (1949) and Nastuk & Hodgkin (1950), following the early work of Overton (1902) on the electrical excitability of nerve and muscle, suggest that an increased permeability toward, and entry of, sodium ions is responsible for the rise of the action potential. If the permeability of the end-plate membrane were increased in a similar way by Ach, the sodium ions on the outside of the muscle fibre would move inward across the membrane and thereby reduce the resting potential.

To test this hypothesis the effect of sodium withdrawal on the depolarizing action of Ach was studied, with the result that Ach was found to produce its local electrical effect even in the complete absence of sodium ions from the

solution bathing the muscle. It becomes necessary then to discuss whether the second mechanism is feasible; that is, whether Ach ions could themselves in very low concentrations produce an electrical discharge of the end-plate membrane.

As an alternative or auxiliary mechanism, the same result could be achieved if Ach were involved in a reaction at the end-plate whereby other ions capable of penetrating the membrane are made available. In any case, if the concentrations and permeabilities of pre-existing diffusible ions are known (notably of potassium and chloride, see Boyle & Conway, 1941), the rate of passage of the 'depolarizing ion' required for a certain steady potential change can be calculated by the method of Goldman (1943). Applying this procedure to the hypothesis that the movement of Ach^+ alone provides the electromotive force for depolarization, the required 'permeability' of the membrane toward Ach can be calculated.

METHOD

Preparation. Various skeletal muscles of the frog, *Rana temporaria*, were used. The m. rectus abdominis and m. sartorius served as the experimental material in preliminary experiments in which mechanical responses alone were recorded. For recording electric potential changes the m. sartorius and m. extensor longus digiti quarti were used. Extreme care had to be taken in the dissection for those experiments in which electrical recording was employed. Visible damage of only a few fibres caused an appreciable negativity to appear at the injured region. All such injured muscles had to be discarded.

There appeared to be no difference in the results obtained with winter and summer frogs. The experiments were carried out at room temperature, varying between 14 and 21° C.

Apparatus. The experimental arrangement employed with the sartorius for the simultaneous recording of electrical and mechanical changes has already been described (Fatt, 1949). In all other experiments the muscle was mounted vertically in a glass chamber so constructed that bathing solutions could be changed through a connexion at the bottom of the vessel. The mechanical response was recorded by attaching the thread tied to the tendon at one end of the muscle to an isotonic lever.

The chamber as used for electrical recording with the extensor digiti IV is shown in Fig. 1. The syringe served to apply a stream of test solution, usually containing Ach, which flowed down the length of the muscle. The leading-off electrodes in contact with the muscle were made of cotton threads stiffened with agar and connected to chlorided spirals of silver. The electrical apparatus consisted of a high impedance input stage, a balanced d.c. amplifier, and a clockwork-driven pen recorder. The stability of the recording system, including the leading-off electrodes, was equivalent to a potential change at the input of not more than 0·2 mV. during the 60 sec. required to take a record.

Experimental procedure. Potentials were recorded by a method similar to that used by Cowan (1936) and Kuffler (1943), with the difference that Ach was applied to the whole muscle rather than locally and that the two recording electrodes were placed, respectively, on the regions of greatest and least end-plate density. (Although there is no recognizable morphological 'end-plate' in the Amphibia, the term is used here in a physiological sense to designate the receptive region of the muscle fibre.) In order to determine the distribution of end-plates a preliminary record was obtained of the potential differences along the surface of each muscle before and after bathing in Ach-containing Ringer solution.

In the case of the sartorius one of the electrodes was kept at the pelvic end while the other was moved from point to point along the surface of the muscle, potential readings being taken every 1–2 mm. An example of the results obtained by this method is illustrated in Fig. 2. The variation

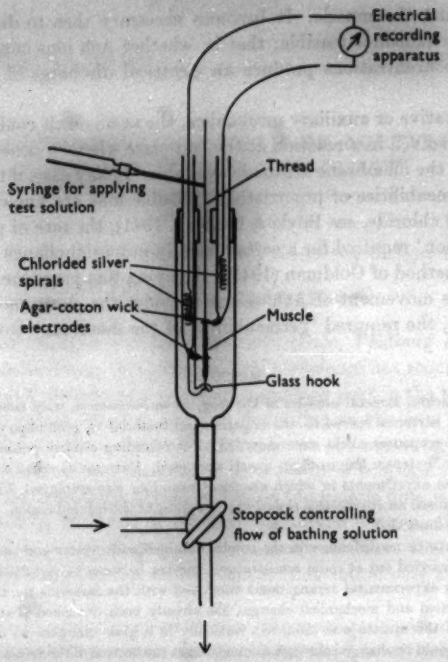

Fig. 1. Apparatus used for measuring potential differences on the m. extensor longus dig. IV.

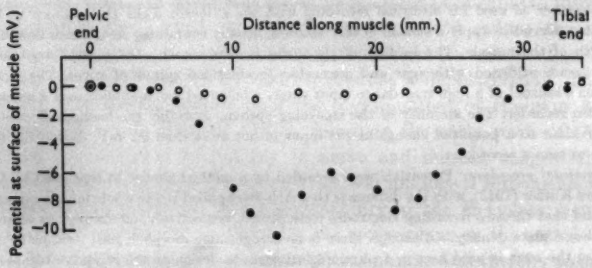

Fig. 2. Potentials measured along a sartorius muscle (○) after immersion in Ringer solution plus eserine sulphate 10^{-5} and (●) a few minutes after adding Ach chloride 10^{-4}. The potentials recorded are those between the moving electrode and the one fixed at the pelvic end. Just before and after each series of readings the bath was flooded and the potential difference between the electrodes found. The change in this potential difference is indicated by a cross-bar above the tibial potential readings.

of depolarization along the muscle agrees with the distribution of neuromuscular junctions as found by other means of electrical recording (Eccles, Katz & Kuffler, 1941) and by histological methods (Pézard & May, 1937).

In the case of the extensor digiti IV, using the chamber of Fig. 1, one electrode was kept at the upper (proximal) end of the muscle, while the other remained near the bottom of the chamber in contact with the solution. With part of the muscle immersed in saline the potential difference recorded was that between the upper end and a point on the muscle at the level of the bathing solution. The solution which initially covered the whole muscle was allowed to flow out of the chamber at an approximately constant rate. With the falling level of the bath serving as a uniformly moving contact on the muscle, the pen recorder gave a continuous tracing of the potential distribution along the muscle surface.

Once having located the position of maximum depolarization, and hence maximum end-plate density, one electrode was placed on that point while the other remained at a region relatively free of end-plates. In both the sartorius and extensor digiti IV the latter position corresponded to the proximal end of the muscle. The time course of the potential change between the two electrodes was then followed as Ach, dissolved in a solution of otherwise the same composition as had previously been bathing the muscle, was applied to the muscle surface. With the sartorius two or three drops of Ach-containing solution were used. The solution rapidly spread along the whole muscle and was diluted by that already present on its surface and in the interstitial spaces. In the case of the extensor digiti IV, to insure that the Ach concentration being used actually reached the end-plates, the drug was applied in a prolonged series of drops which, flowing down the muscle, mixed with and gradually replaced the solution adhering to its surface. Droplets, formed at the points where the electrodes made contact with the muscle, served as reservoirs which maintained a constant composition of the solution bathing the fibres.

At all times except when potentials were being recorded the muscle was kept immersed in solution.

Solutions. Unless otherwise noted in the sodium-free solution, NaCl (0·675%) was replaced by 3·70% glucose. The other constituents of frog-Ringer solution remained unchanged: 2·0 mM. K^+, 1·8 mM. Ca^{++}, 1·0 mM. phosphate, and Cl^- to make up the anion balance. The pH was about 6·8. In some experiments on the sartorius eserine sulphate in a concentration of 10^{-5} was used; in most of those on the extensor digiti IV prostigmine bromide in a concentration of 10^{-6} was used.

An internal micro-electrode as described by Graham & Gerard (1946) and Hodgkin & Nastuk (1949) was used to obtain information on the effect of the removal of sodium ions on the resting potential. Frog sartorii served as the experimental material.

RESULTS

Sodium ions and mechanical response

In preliminary experiments on the rectus abdominis the mechanical response was observed which could be evoked by Ach chloride in concentrations of 10^{-6} to 10^{-5}. Ach was applied first after bathing in normal Ringer solution and again after bathing in sodium-free solution. After soaking the muscle in the sodium-free medium for 40 min. it was found that Ach no longer elicited a contracture. This result was confirmed on the sartorius, and it agrees with the findings of Wachholder & Matthias (1933), Cicardo (1938) and others in various frog-muscle preparations.

That the removal of sodium did not abolish the contractility of the muscle was shown by the fact that application of potassium chloride (20–50 mM.) still produced a contracture. With the rectus abdominis the KCl contractures did not differ markedly whether sodium was present in the solution or not.

With the sartorius the pronounced initial twitching, which is normally produced by excess potassium, was abolished when sodium had been removed leaving only the contracture response. Direct electrical stimulation was, on the other hand, very ineffective in producing a mechanical response in the absence of sodium. Using six pairs of stimulating electrodes along the length of a sartorius muscle, condenser discharges (RC product = 1 msec.) could produce only very weak local contractions. Similar results were obtained by Overton (1904) with muscles in sodium-free solutions provided some calcium salts were present.

Sodium ions and acetylcholine depolarization

Although these experiments showed that the presence of sodium was necessary for the appearance of the mechanical response to Ach, such observations alone are inconclusive in that they cannot reveal the direct action of Ach. Kuffler (1943) showed that in the concentrations used here Ach depolarizes only the regions of the muscle fibre around the neuromuscular junction. The removal of sodium by stopping propagation would prevent the spread of depolarization farther along the fibre, and it is therefore conceivable that an insufficient length of each fibre might have been depolarized to give an observable mechanical response. The action of Ach would be similar to an electrical stimulus acting at one or a few points along each fibre. On the other hand, if the entire fibre is depolarized, as by potassium chloride, there is a visible contraction even in the absence of a propagating mechanism.

It was therefore necessary to study the action of Ach more directly, and to record electric potential changes at the end-plate focus of the muscle. When Ach in a concentration of $1–2 \times 10^{-6}$ was applied to a muscle previously soaked in Ringer solution the focal region became depolarized (see Fig. 3*a*). Simultaneously, propagated action potentials were set up which, though not faithfully reproduced by the recording apparatus, complicated the record by being led off at both electrodes. After propagated impulses had ceased the true level of depolarization could be determined. The depolarization was maintained over a period of minutes, its amplitude depending on the Ach concentration. In the absence of anti-cholinesterases it is unlikely that all the end-plates would be reached, or, at any rate, unfformly acted on. The depolarization at an end-plate focus of the sartorius produced by Ach in the stated concentrations amounted under these circumstances to 5–10 mV.

A difficulty was encountered when the muscles were soaked in sodium-free solutions. Although the surface of uninjured muscles was normally, i.e. after immersion in Ringer solution, at almost uniform potential (differences not exceeding 1 or 2 mV., see Fig. 2), when NaCl had been replaced by glucose or sucrose, potential differences of up to 10 mV. appeared, which varied in an irregular manner along the muscle surface and were very unsteady. Similar effects have previously been described by Fenn (1931), who suggested that they

might be responsible for changes in the mechanical state of the muscle. With the sartorius these potential differences usually vanished after prolonged soaking in the sodium-free solution (1–3 hr.). Other investigators (Lorente de Nó, 1947; Nastuk & Hodgkin, 1950) have been able to overcome such difficulties in measurements of the resting potential of nerve and muscle by using choline chloride, rather than a non-electrolyte, to replace NaCl. But choline has itself a depolarizing action on end-plates, and was therefore not suitable, nor has any other 'inert' cation been found which could be used.

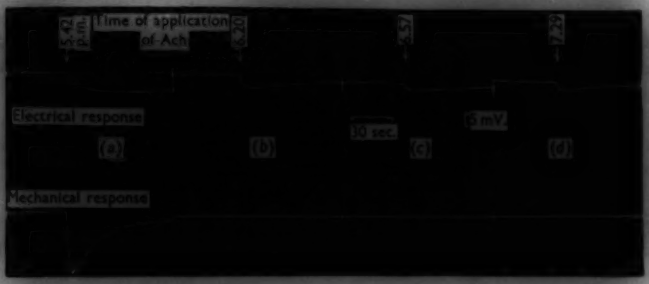

Fig. 3. Electrical and mechanical responses of a sartorius muscle to Ach 2×10^{-6}: (*a*) after bathing in Ringer solution; (*b*) after bathing in a mixture of 5% Ringer solution and 95% sodium-free solution; and (*c*) and (*d*) after bathing in sodium-free solution. The muscle was immersed in the solutions for about 35 min. before Ach was applied. Each test with Ach was immediately preceded by application of some of the bathing solution. Individual spikes do not appear in the electrical record because of the slow response of the recording instrument.

After sartorius muscles had been immersed in sodium-free solution long enough for the initial potential differences to subside, Ach still evoked a depolarization of the end-plate focus. The maximum depolarization was, however, only about 50% of that occurring in the presence of sodium, in spite of the fact that the sodium-free solution had a much lower conductivity and therefore caused less external shunting of the true membrane depolarization. The depolarization was considerably increased when only 5–10% of the NaCl contained in Ringer solution was added to the sodium-free medium. This is seen in Fig. 3, where Ach was applied in a concentration of 2×10^{-6} to a sartorius muscle after soaking (*a*) in Ringer solution, (*b*) in a solution containing 5·8 mM. sodium (i.e. 5% of the sodium content of Ringer solution), and (*c*) and (*d*) in sodium-free solution.

After each Ach application the solution in the bath was changed and the muscle allowed to equilibrate in the new solution for about 35 min. before another test. Immediately preceding each test with Ach a few drops of the bathing solution were applied to the muscle. Although in the experiment illustrated the initial potential gradients occurring in electrolyte-deficient solutions subsided rapidly, there is a small deflexion of the electrical record at the beginning of test (*b*)

caused by a shunting of the potential difference between the points under the two electrodes. The two tests (*c*) and (*d*) in sodium-free solution show that no further change attributable to a continued loss of sodium took place following the initial periods of soaking.

When, instead of adding sodium, the calcium ion content of the sodium-free solution was increased to three times that in Ringer solution, or both the calcium and potassium content raised (calcium three times, potassium two times), a decrease in the Ach-depolarization was observed. This is of interest because it confirms that the potentiation of the Ach effect was specifically due to sodium ions. The reduction of the recorded depolarization by added calcium or calcium plus potassium was probably largely the effect of increased shunting, although a specific action of the ions cannot be excluded. Adding then small amounts of sodium to the solution of high calcium and potassium content caused an increase of the depolarization.

Removal of sodium not only reduced the maximum recorded effect of Ach, but also necessitated two to three times higher Ach concentrations in order to attain this maximum.

It is clear from the foregoing that the presence of sodium is not essential for the occurrence of a depolarization at the end-plate, but that it somehow serves to potentiate the action of Ach. This might be explained if sodium ions are normally responsible for the setting up of a 'local response' in the neighbourhood of the end-plate, that is, for an active reinforcement of the depolarization produced by Ach. Hodgkin & Katz (1949) have suggested that sodium ions mediate in the excitatory process by entering the fibre as it becomes depolarized, thereby intensifying the initial potential change and causing the depolarization to spread to a greater distance than would be possible simply by means of the passive cable properties of the fibre. Because of the scattered distribution of end-plates in a muscle a portion of the observed potential change must be due to spread of the depolarization from end-plates situated a short distance away from the recording lead. Sodium ions would therefore cause an increased potential change in two ways: first, by augmenting the depolarization immediately at the end-plate; and, secondly, by allowing more end-plates to contribute, due to an increased spread of depolarization.

Relation between depolarization and acetylcholine concentration

Having found that sodium ions reinforce, but are not essential for the electrical action of Ach, further experiments were made in the absence of sodium. The electrical changes so recorded give probably a simpler and more direct indication of the reaction between Ach and the end-plate membrane.

To obtain consistent quantitative measurements some improvements in the stability of the preparation were necessary. The m. extensor digiti IV provided certain advantages. Potential differences which occurred when preparations were immersed in sodium-free solutions subsided more quickly in this small

muscle, while the individual fibres survived as well as in the sartorius. The results became also more consistent when prostigmine (as the bromide salt) in a concentration of 10^{-6} was added to the solutions. In this way, with an anti-cholinesterase present, Ach was more likley to exert a uniform effect throughout the muscle, and to reach the deeper as well as the superficial fibres. As a result the observed potential changes became appreciably larger.

With these improvements it became possible to measure the relation between Ach concentration and depolarization. Muscles were bathed in sodium-free solutions, and Ach was applied in various concentrations at intervals of 15–20 min. In Fig. 4 tracings of several records obtained on a single muscle are

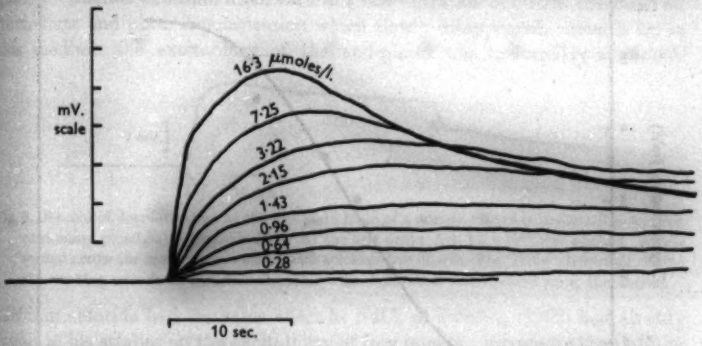

Fig. 4. Tracings of electrical records showing the depolarization at the end-plate focus of m. extensor digiti IV when applying various concentrations of Ach. The muscle had previously been bathed in sodium-free solution containing prostigmine bromide 10^{-6} and was kept in that solution between tests. The Ach concentration is indicated on each trace (1 μmole/l. corresponds to a concentration for Ach chloride of $1{\cdot}82 \times 10^{-7}$).

superimposed. The Ach concentration is shown on each trace in μmoles/l. The concentrations range from one which produced a barely detectable depolarization to one which gave a maximum effect. Of six muscles examined in this way all gave essentially similar results. The relative changes in depolarization were the same within the same concentration range. The absolute value of depolarization, however, varied from muscle to muscle, depending, no doubt, on the local density of end-plates.

An interesting phenomenon was that with high concentrations of Ach the initial depolarization was not maintained. This had also been observed, although much less marked, in the presence of sodium. Further addition of Ach, following the decline of depolarization, did not restore it to its original value. In fact, long periods of washing in Ach-free solution were necessary for the depression of the end-plate sensitivity to pass away, and even then

after concentrations exceeding 30 μmoles/l. complete recovery was usually not obtained. Measurements made with such large doses, although not wholly reproducible, were necessary as they indicated the size of the maximum depolarization which could be obtained.

In Fig. 5 the depolarization reached after each application of Ach is plotted against Ach concentration. The results have been taken from the experiment illustrated in Fig. 4. For convenience Ach concentration is plotted on a logarithmic scale. The exact shape of the curve is probably of no special significance; it is drawn to a maximum at concentrations which were found to

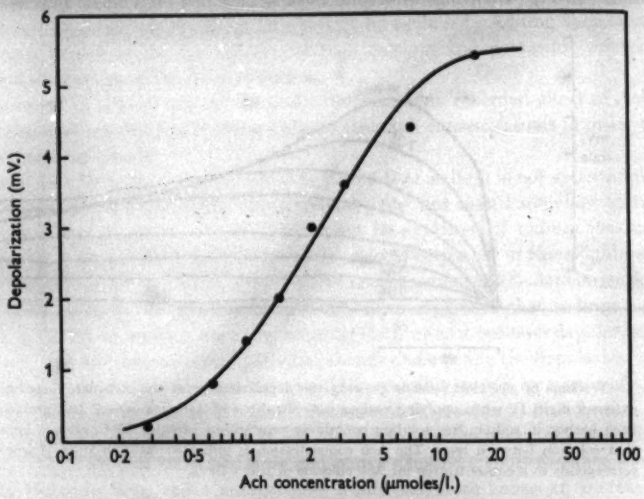

Fig. 5. Height of depolarization produced in the muscle of Fig. 4 plotted against Ach concentration. Ach concentration is plotted on a logarithmic scale.

produce a maximum depolarization in several similar experiments. What is important is the range of concentrations in which Ach begins to be effective on the end-plates. One-half its maximum effect occurs at a concentration of about 2 μmoles/l.

Some comment is required on the recovery of membrane potential when Ach was removed from the bath. The time course is illustrated in Fig. 6, where a muscle was washed with Ach-free solution 50 sec. after the application of Ach in a concentration of $1{\cdot}2 \times 10^{-6}$. Depolarization was only partly reversed immediately after the removal of Ach. There was a residual effect which required prolonged washing and then gradually disappeared, and this was found at all Ach concentrations used.

Bathing the muscle in D-tubocurarine chloride in a concentration of 10^{-5} greatly reduced the depolarizing effect of Ach. It is interesting that eserine sulphate in a concentration of 10^{-4} produced the same effect. This observation agrees with those of Eccles & MacFarlane (1949), who found that eserine, even in moderate concentrations (i.e. concentrations necessary for complete cholinesterase inhibition), produces a depression of end-plate potentials. With the concentrations used in the present experiments neither curarine nor eserine had a depolarizing action themselves.

As substitutes for NaCl, glucose, sucrose, choline chloride, LiCl, and mixtures of glucose and $CaCl_2$ were tried. Sucrose gave in all respects the same results as glucose. Choline chloride, when used in a concentration of 0·12 M. produced an immediate end-plate depolarization which slowly disappeared. After 2 hr. in this medium the excitability of the end-plates was irreversibly abolished.

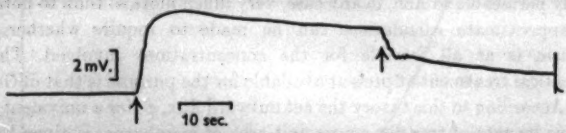

Fig. 6. Record of depolarization at the end-plate focus of a muscle bathed in sodium-free solution containing prostigmine bromide 10^{-6}. At the first arrow Ach $1{\cdot}2 \times 10^{-4}$ was applied. At the second arrow the muscle was washed with solution free of Ach. The 'jerky' deflexions during the washing arise from transient shunting when individual drops flowed along the muscle.

Lithium chloride had the same effect as NaCl, as Overton (1902) had already found in his studies on the excitability of frog muscle. Increasing the $CaCl_2$ in the sodium-free solution to 22 mM., while reducing the initial instability of potential, caused an irreversible decrease in the excitability of the muscle, again confirming Overton (1904).

DISCUSSION

The experiments described in this paper rule out the suggestion that Ach might act primarily by making the end-plate more permeable to sodium ions. Sodium is, nevertheless, important for the normal process of neuromuscular transmission in that it amplifies the initial local depolarization and enables it to lead to a propagated potential change and associated contraction. As was shown for direct electrical stimulation, only very weak local contractions can be set up in the absence of sodium by depolarizing at a few points, while when the muscle fibre membrane is depolarized along its entire length, as by potassium chloride, the contraction is much the same whether or not sodium ions are present.

Since the movement of sodium ions is not the immediate cause of the end-plate depolarization the next possibility to consider is whether Ach^+ ions could themselves discharge the membrane to the observed extent. The only specialized property of the end-plate region of the muscle fibre necessary for this mechanism

would be a specifically high permeability to Ach. For the membrane potential to be reduced by any given amount a certain rate of flow of electric charge across unit area of membrane must be provided by the depolarizing agent. When Ach^+ ions move inward the electric potential difference across the membrane which normally maintains the high internal potassium concentration (see Ussing, 1949) is reduced, and an outward flow of potassium ions must occur. For every observed steady level of membrane potential the inward flow of Ach must equal the outward flow of potassium. Chloride ions need not be considered, as in the sodium-free solution their concentration was reduced to only 4 mM. compared to 120 mM. in Ringer solution.

If Ach is to be an effective depolarizing agent in concentrations of only a few μmoles/l., while diffusible potassium ions are present within the fibre in a concentration of about 100 mmoles/l., the membrane will have to be exceedingly permeable to Ach, in any case, very much more so than to potassium. Some approximate calculations can be made to inquire whether such a mechanism is at all feasible for the concentrations involved. The only mathematical treatment at present available for the purpose is that of Goldman (1943). According to this theory the net outward flux, ϕ, for a univalent cation, defined as its rate of transfer across unit area of membrane, is given by

$$\phi = -P\frac{EF}{RT}\frac{c_o - c_i e^{-EF/RT}}{1 - e^{-EF/RT}}, \qquad (1)$$

where P is a permeability coefficient for the ion due to osmotic forces alone; E is the membrane potential; c_o and c_i are the outside and inside concentrations of the ion; and R, T and F are the gas constant, the absolute temperature and the faraday, respectively. A similar equation with c_o and c_i reversed applies for anions. (For the assumptions involved in its derivation see Goldman, 1943; and also Hodgkin & Katz, 1949).

The relationships expressed in equation (1) may be used to calculate the increased outward flux of potassium as the membrane is depolarized in the absence of sodium. If the end-plate region is assumed to have a potassium permeability equal to that of other regions of the fibre, the permeability coefficient P can be obtained from studies in which radioactive tracers have been used. From the results of the 'soaking in' experiments of Harris & Burn (1949), together with a membrane potential in Ringer solution of 87 mV. (Hodgkin & Nastuk, 1949), the permeability coefficient P for potassium ions is calculated to be $2{\cdot}7 \times 10^{-7}$ cm./sec. By means of capillary microelectrodes the membrane potential was observed after the removal of sodium and was found to be rather less than in Ringer solution. The potentials were highly variable, ranging between 55 and 80 mV. On returning to Ringer solution after 2 hr. the membrane potential recovered its original value of 80–90 mV. The fact that in the sodium-free solution the membrane potential was less than

for a potassium concentration cell, i.e. $RT/F \log_e c_i/c_o$, indicates that the fibres are losing potassium. This leak can be allowed for in the mathematical treatment by assigning to c_o a value higher than the true external potassium concentration. In any case, the initial size of the resting potential has, over a wide range, only a very small effect on the relations calculated here. In Fig. 7 a curve is shown, calculated from equation (1), which relates membrane depolarization to extra potassium flux resulting from the depolarization.

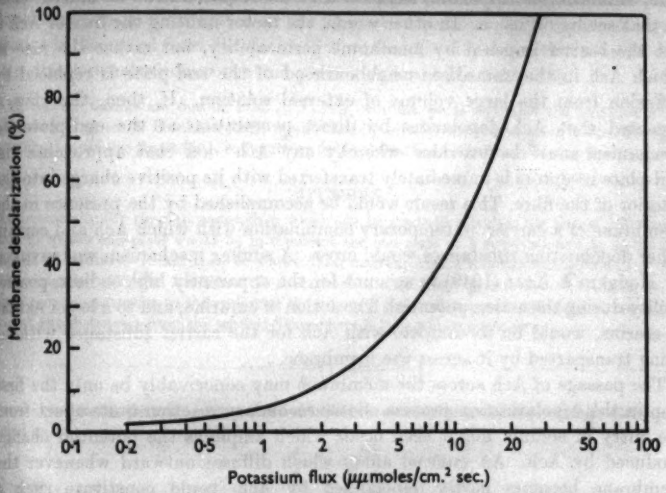

Fig. 7. Membrane depolarization, in percentage, plotted against outward potassium flux calculated from equation (1). Potassium flux is plotted on a logarithmic scale.

In order to apply this curve to the experimental findings some assumption must be made which will permit the actual magnitude of membrane depolarization to be derived from the observed potential changes. The simplest assumption is that the maximum effect observed with high Ach concentrations corresponds to a complete depolarization of the end-plate membrane.

Since the inward flux of Ach at each steady level of depolarization must be equal to the extra outward flux of potassium, the relation of Ach flux to Ach concentration can be obtained by comparing Figs. 5 and 7. The relative ordinate scales must first be adjusted so that maximum depolarization in Fig. 5 corresponds to 100% depolarization in Fig. 7. It is then seen that over the region where the curves are determined with greatest accuracy, i.e. at about one-half depolarization, the ratio of inward Ach flux to external Ach concentration is approximately 5×10^{-3} cm./sec. This quantity represents a form of permeability

coefficient for Ach. It should be noted, however, that it is based only on the concentration existing in the solution around the muscle and not on the true difference in concentration across the membrane. With so high a ratio of flux to concentration it must be inquired whether diffusion of Ach in the solution external to the end-plate membrane would be sufficiently rapid to allow the proposed mechanism to operate. In order to form a quantitative estimate a rough guess must be made regarding the size and shape of the end-plate. This estimate (see Appendix) suggests that the required ratio is, indeed, close to that set by diffusion. In other words, the factor limiting the flux of Ach is not the barrier imposed by membrane permeability, but rather the rate at which Ach in the immediate neighbourhood of the end-plate is replaced by diffusion from the large volume of external solution. If, then, the view is accepted that Ach depolarizes by direct penetration at the end-plates, a mechanism must be provided whereby any Ach^+ ion that approaches the end-plate receptors is immediately transferred with its positive charge into the interior of the fibre. This result would be accomplished by the presence in the membrane of a carrier, in temporary combination with which Ach and certain other depolarizing substances would cross. A similar mechanism was invoked by Hodgkin & Katz (1949) to account for the apparently high sodium permeability during the action potential. The action of curarine, and to a lesser extent of eserine, would be to compete with Ach for the carrier substance without being transported by it across the membrane.

The passage of Ach across the membrane may conceivably be only the first step in the depolarization process. Some secondary reaction quite apart from the entry of sodium might then occur which amplifies the potential change produced by Ach. An internal anion which diffuses outward whenever the membrane becomes partly depolarized by Ach, could constitute such a mechanism.

For more direct evidence on the processes occurring at the end-plate a better experimental preparation will have to be used. Small groups of muscle fibres were dissected out of the m. adductor magnus and used in a modification of the procedure described for exploring the extensor digiti IV. Depolarizations around end-plates were recorded, but the fibres were in poor condition and did not survive soaking in the different solutions. The microelectrode technique was also tried, but certain difficulties in the procedure have so far precluded its use in more critical experiments.

SUMMARY

1. The origin of the depolarization which acetylcholine produces at the motor end-plates of muscle fibres is discussed. One of the suggested mechanisms which requires an increase of the sodium permeability of the end-plate membrane is subjected to experimental test.

2. When frog muscles are kept in sodium-free solutions, Ach in concentrations of 10^{-7} and 10^{-6}, although not eliciting any visible mechanical response, still produces a local depolarization at the end-plate.

3. The presence of sodium increases the depolarization produced by Ach and leads to the setting up of propagated impulses. Sodium in concentrations below that necessary for propagated impulses already exerts a marked effect on the magnitude of the local depolarization.

4. The quantitative relation between Ach concentration and end-plate depolarization is investigated in the absence of sodium. Its theoretical significance is discussed from the point of view that the depolarization may be due to a direct penetration of the end-plate membrane by Ach^+ ions.

My sincere thanks are due to Prof. A. V. Hill, who has made available the facilities of his laboratory, and to Dr Bernhard Katz, whose friendly interest and advice have made this work possible. I must also thank Mr J. L. Parkinson for his skilled assistance in assembling the apparatus.

APPENDIX

Mathematically the simplest assumption which can be made to calculate the rate of diffusion of Ach up to the end-plate would be to represent the end-plate as a spherical sink in an infinite volume of solution. It can then be shown that, starting with a uniform concentration c^0 of a substance with diffusion coefficient D throughout the infinite volume, and maintaining at all times zero concentration at the surface of the sphere of radius ρ^0, the concentration c at any time t and at any distance ρ from the centre of the sphere ($\rho > \rho^0$) will be given by

$$c = c^0 - \frac{\rho^0 c^0}{\rho}\left(1 - \operatorname{erf}\frac{\rho - \rho^0}{2\sqrt{Dt}}\right). \tag{2}$$

From equation (2) the ratio of flux across the surface to concentration at an infinite distance is found to be

$$\frac{\phi}{c^0} = \frac{D}{\rho^0} + \sqrt{\frac{D}{\pi t}}. \tag{3}$$

(Cf. Carslaw & Jaeger (1947) for the derivation of an equation analogous to equation (2) applied to heat conduction in solids.)

For Ach the condition that the concentration at the end-plate surface remains zero will be fulfilled if Ach is immobilized or removed in some way as rapidly as it enters the fibre so that it cannot diffuse back across the membrane. From the equivalent conductance found for Ach^+ in dilute solutions (29 Ω^{-1} cm.^{2}) the diffusion coefficient is calculated to be $7{\cdot}6 \times 10^{-6}$ cm.^{2}/sec. The selection of a value for ρ^0 can only be made very approximately. If ρ^0 is taken as of the same order as the radius of the fibre, say 5×10^{-3} cm., a steady state of Ach flux will be reached with ϕ/c^0 equal to $1{\cdot}5 \times 10^{-3}$ cm./sec.

If this figure is compared with the theoretically required Ach 'permeability' of the end-plate membrane, i.e. 5×10^{-3} cm./sec. (p. 419), it is seen, as far as the accuracy of this procedure will allow, that the two are of the same order of magnitude. Thus, while the limit set by diffusion in the external medium may not make the theory altogether untenable, it does become the factor deciding the rate at which Ach will pass the end-plate membrane under the conditions of the experiments described in this paper.

REFERENCES

Boyle, P. J. & Conway, E. J. (1941). *J. Physiol.* **100**, 1.
Brown, G. L. (1937). *J. Physiol.* **89**, 220.
Brown, G. L., Dale, H. H. & Feldberg, W. (1936). *J. Physiol.* **87**, 394.
Carslaw, H. S. & Jaeger, J. C. (1947). *Conduction of Heat in Solids*, p. 209. Oxford University Press.
Cicardo, V. H. (1938). *Rev. Soc. argent. Biol.* **14**, 370.
Cowan, S. L. (1936). *J. Physiol.* **88**, 3*P*.
Dale, H. H., Feldberg, W. & Vogt, M. (1936). *J. Physiol.* **86**, 353.
Eccles, J. C., Katz, B. & Kuffler, S. W. (1941). *J. Neurophysiol.* **4**, 362.
Eccles, J. C. & MacFarlane, W. V. (1949). *J. Neurophysiol.* **12**, 59.
Fatt, P. (1949). *J. Physiol.* **109**, 10*P*.
Fenn, W. O. (1931). *Amer. J. Physiol.* **97**, 635.
Goldman, D. E. (1943). *J. gen. Physiol.* **27**, 37.
Graham, J. & Gerard, R. W. (1946). *J. cell. comp. Physiol.* **28**, 99.
Harris, E. J. & Burn, G. P. (1949). *Trans. Faraday Soc.* **45**, 508.
Hodgkin, A. L. & Katz, B. (1949). *J. Physiol.* **108**, 37.
Hodgkin, A. L. & Nastuk, W. L. (1949). *J. Physiol.* **108**, 42*P*.
Katz, B. (1942). *J. Neurophysiol.* **5**, 169.
Kuffler, S. W. (1943). *J. Neurophysiol.* **6**, 99.
Lorente de Nó, R. (1947). *Stud. Rockefeller Inst. med. Res.* **131**.
Nastuk, W. L. & Hodgkin, A. L. (1950). *J. cell. comp. Physiol.* **35**, 39.
Overton, E. (1902). *Pflüg. Arch. ges. Physiol.* **92**, 346.
Overton, E. (1904). *Pflüg. Arch. ges. Physiol.* **105**, 176.
Pézard, A. & May, R. M. (1937). *C.R. Soc. Biol., Paris*, **124**, 108.
Ussing, H. H. (1949). *Physiol. Rev.* **29**, 127.
Wachholder, K. & Matthias, F. (1933). *Pflüg. Arch. ges. Physiol.* **232**, 159.

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URINARY EXCRETION OF PHOSPHATE IN MAN AND THE CAT

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The dominating factor controlling phosphate excretion when the concentration of this ion in the blood plasma has been artificially raised is, both in man (Ollayos & Winkler, 1943) and in the dog (Harrison & Harrison, 1941; Pitts & Alexander, 1944), this concentration itself; since the tubule cells can only reabsorb a limited amount of phosphate, the clearance rises as the concentration in the plasma is increased. When extra phosphate is not given, factors other than variation in the concentration in the plasma can be observed to affect the clearance. In fact, the variation in such concentration is so small under normal conditions that much can still be learned by following the changes in rate of phosphate excretion in place of the more complex determination of changes in absolute clearance values. Thus Haldane (1921) observed a large increase in phosphate excretion when the body had been acidified by ingestion of ammonium chloride. Havard & Reay (1926) noted a similar effect after severe exercise, the increased excretion long outlasting the very small and fleeting increase in plasma phosphate concentration; the increase in phosphate output under these conditions was confirmed by Eggleton (1942). The mechanism of this action in the kidney is not yet understood.

Attempts to correlate the changes observed in phosphate output under other experimental conditions have led to no very definite results. Most workers are agreed that rate of urine flow is not concerned, though Dean & McCance (1948) have recently revived the suggestion on finding a significant correlation between the two in newborn infants. The probable explanation of this will be discussed later. In view of the fact that there is a limit to the amount of phosphate which can be reabsorbed from the tubules, it would seem highly probable that many changes in phosphate excretion normally observed might be explained as due to variation in the amount of phosphate filtered: a change in glomerular filtration rate (G.F.R.) would have a similar effect to that produced by a change in plasma phosphate concentration. Experiments were designed, therefore, to induce relatively large variations in G.F.R., in urine pH

and in rate of flow in order to see with which variable, if any, the phosphate output was correlated.

Opportunity also arose to study the phosphate clearance in cats and to determine the maximum amount which could be reabsorbed by the tubule cells (*Tm* value) and the effect of various experimental procedures upon it.

METHODS

Changes in creatinine output in man—an indication of changes in G.F.R. (Eggleton, 1949)—in rate of urine flow and in urine pH were induced in most experiments by ingestion of ethyl alcohol. This was taken on an empty stomach in a dose of 30–60 g. (diluted to 200–300 c.c.) and urine samples collected at 20–30 min. intervals. In a few experiments nicotine was injected intravenously after either water or alcohol had been ingested, and in a few others tea was taken in place of alcohol. No attempt was made to regulate the fluid balance of the body, since the magnitude of the diuretic response was not being studied.

pH was determined immediately the sample had been collected, by the use of indicators. Creatinine and phosphate were determined by use of a photoelectric absorptiometer. Details of the methods used and of the experimental procedure with cats has been described fully elsewhere (Eggleton & Habib, 1949). In the cats, phosphate clearance was studied when the kidney was exposed to the action of thiosulphate or of *p*-aminohippuric acid (PAH) and also when the plasma phosphate concentration was artificially raised by injection or infusion of sodium phosphate solution at pH 7·4. These experiments were all performed under nembutal anaesthesia.

PART I. MAN

Results

Each urine sample was compared with the preceding one in respect of changes in creatinine output, phosphate output and pH. Thus, each experiment yielded five to ten such comparisons. From the twenty experiments performed on eleven individuals, 117 such comparisons were made. In thirty-nine of these, an increase in phosphate output accompanied an increase in filtration rate coinciding with a fall in pH or vice versa. The results of an experiment in which this double relationship predominated are given in Fig. 1. At only one point does it fail, the phosphate output then being related to pH.

In seventeen other comparisons, this latter relationship was observed; that is, the phosphate output rose with fall in pH or vice versa, although the G.F.R. remained unchanged or moved in contrary direction. In forty-one comparisons, however, the relationship between phosphate output and G.F.R. predominated. The results of an experiment shown in Fig. 2 demonstrate this effect.

In 20 observations, that is 17% of the total, there was no apparent relationship between the three variables: a fall in phosphate output occurred despite an increase in G.F.R. and fall in pH or a rise accompanied a decrease in G.F.R. and rise in pH. Since the most important factor regulating phosphate output under normal conditions is its concentration in the plasma, and since a small change in this concentration has a pronounced effect on the clearance, it would seem highly probable that this factor is responsible for these anomalous observations. The results of an experiment depicted in Fig. 3 illustrate the

point. Unlike the course of events in most experiments, there was a remarkable decline in phosphate output throughout, which could most reasonably be attributed to change in plasma phosphate concentration, but even here the relationship with G.F.R. was not entirely obscured, although the magnitude of each fall was undoubtedly affected by the underlying factor responsible for the steady downward drift in its output.

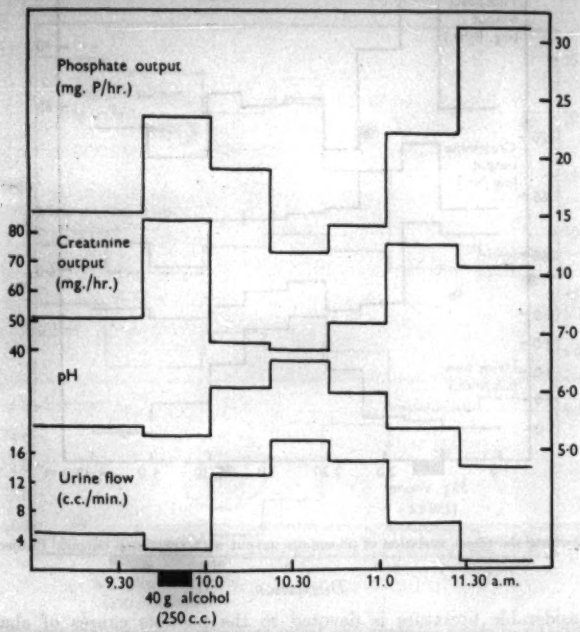

Fig. 1. Showing the variation in phosphate output: (*a*) directly with creatinine output, and (*b*) inversely with urine pH. Subject *A*.

A statistical analysis of the results indicates that the relationship between phosphate output and urine pH is not significant. The 39 observations in which pH might have been the dominant factor plus the 17 observations in which the relationship was directly observed adds to 56. On the laws of chance a value of $58\frac{1}{2}$ ($117 \div 2$) might be expected. Similarly, with rate of urine flow: changes in phosphate output and rate of flow ran parallel in 56 observations and in contrary direction in 61. Only in the case of the relationship with G.F.R. can any significance be attached to the results. Here, phosphate output varied

with G.F.R. in a total of 80 (39+41) observations in place of the expected 58½ on the law of chance. The value of $t=3{\cdot}98$* which can be calculated from the results is highly significant; the probability of such a result by chance is only 1 in 20,000.

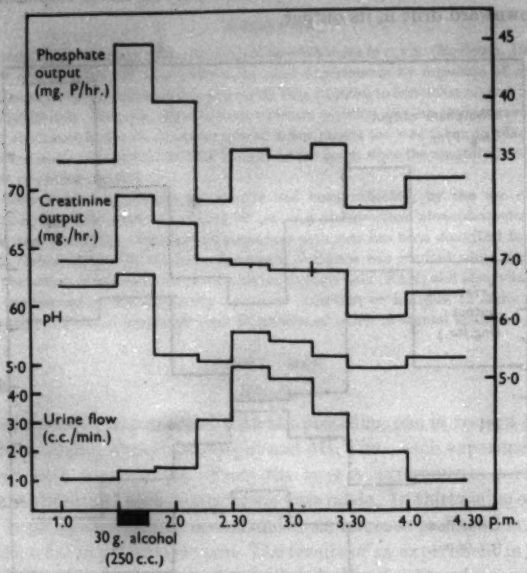

Fig. 2. Showing the direct variation of phosphate output with creatinine output. Subject *H*.

Discussion

A considerable literature is devoted to the possible causes of changing phosphate output in man, but hitherto no attention has been paid to the effect of changes in G.F.R. Apart from the well-established fact that acidosis of the body results in an increased output, little but negative evidence has been obtained. Most workers agree, and our own findings confirm, that change in rate of urine flow has no effect. The opposing results of Dean & McCance (1948) in experiments on newborn infants is open to quite a different interpretation, for McCance & Young had previously shown (1941) that in these, the G.F.R. varies with rate of urine flow; it is probable, therefore, that here also the

* Derived from a simplified formula

$$\frac{80-58\frac{1}{2}}{\frac{1}{2}\sqrt{117}}=3{\cdot}98.$$

causative factor is the change in G.F.R., the relationship with rate of urine flow being only an indirect one.

The lack of any correlation between urine pH and phosphate output might seem at first sight surprising, since such a correlation exists when the body is made acidic. The fall in pH which is often induced by ingestion of alcohol is, however, accompanied by a fall in the total buffer output similar to that observed after injection of hypertonic solutions (Eggleton, 1947). The main

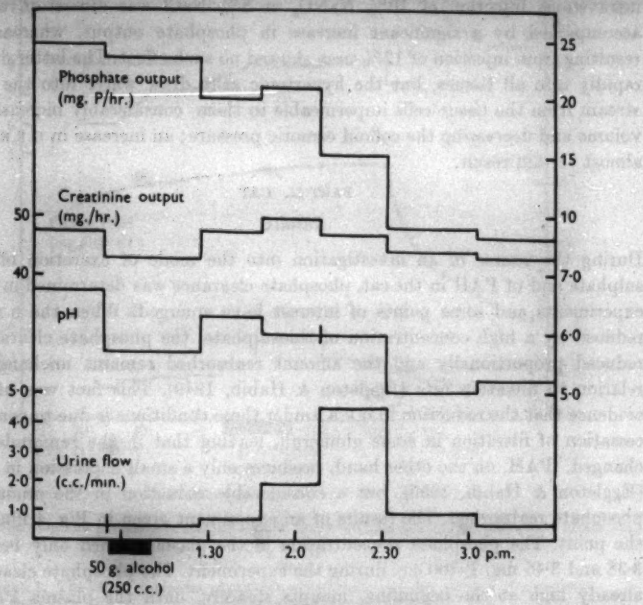

Fig. 3. Showing the variation of phosphate output with creatinine output on a background of steadily diminishing phosphate output. Subject *E*.

buffers in the urine are bicarbonate and phosphate, and the results now obtained suggest that the variation in pH under these conditions is mainly due to varying reabsorption of bicarbonate, the phosphate output showing no consistent change.

The observed relationship between phosphate output and G.F.R., on the other hand, is not surprising. It has been found both in man (Barclay, Bray & Cooke, 1944) and in the dog (Pitts & Alexander, 1944) that phosphate reabsorption has a *Tm* value. Unless the urine is phosphate-free, therefore, any increase in the amount filtered, whether due to a rise in plasma phosphate

concentration or to an increase in G.F.R. will automatically increase the amount excreted.

The results of Hendrix & Calvin (1925) on the effects of intravenous injection of hypertonic solutions on phosphate output can also be interpreted in this light, though less directly. The experiments were of longer duration, a diuretic period of 4 hr. being compared with a control period of 48 hr. and a post-diuretic period of 20 hr., in unanaesthetized dogs. The diuresis resulting from intravenous injection of 10% $NaNO_3$ or 8% NaCl was almost invariably accompanied by a significant increase in phosphate output, whereas that resulting from injection of 12% urea showed no such effect. The latter diffuses rapidly into all tissues, but the hypertonic salts draw water into the blood stream from the tissue cells impermeable to them, considerably increasing its volume and decreasing the colloid osmotic pressure; an increase in G.F.R. is an almost certain result.

PART II. CAT

Results

During the course of an investigation into the mode of excretion of thiosulphate and of PAH in the cat, phosphate clearance was determined in many experiments and some points of interest have emerged. When the G.F.R. is reduced by a high concentration of thiosulphate, the phosphate clearance is reduced proportionally and the amount reabsorbed remains unchanged in relation to filtration rate (Eggleton & Habib, 1949). This fact was used as evidence that the reduction in G.F.R. under these conditions is due to complete cessation of filtration in some glomeruli, leaving that in the remainder unchanged. PAH, on the other hand, produces only a small depression in G.F.R. (Eggleton & Habib, 1950), but a considerable reduction in the amount of phosphate reabsorbed. The results of an experiment given in Fig. 4 illustrate the point. The phosphate concentration in the plasma varied only between 3·38 and 3·46 mg. P/100 c.c. during the experiment. The phosphate clearance, already high at the beginning, mounts steadily, until the plasma PAH is allowed to fall, and then drops rapidly as this concentration diminishes. At first sight, it might appear that change in phosphate clearance is related to change in rate of urine flow, but there is no correlation between the magnitudes of the changes in the two: the last low clearance was obtained at the same rate of flow as that occurring in the first sampling period. Taking the experiments as a whole, there is, in fact, no hint of any relationship between the two.

In the majority of experiments the plasma phosphate concentration tended to rise, sometimes considerably, and it became apparent that the amount reabsorbed varied directly with this concentration. As the concentration increased, so also did the amount excreted in the urine and also the amount reabsorbed. A comparison of results from different experiments showed a

similar relationship to exist, the amount reabsorbed being fairly constant at any given plasma phosphate concentration. In Table 1 are given the average results of the normal values (seventeen experiments), those obtained under the

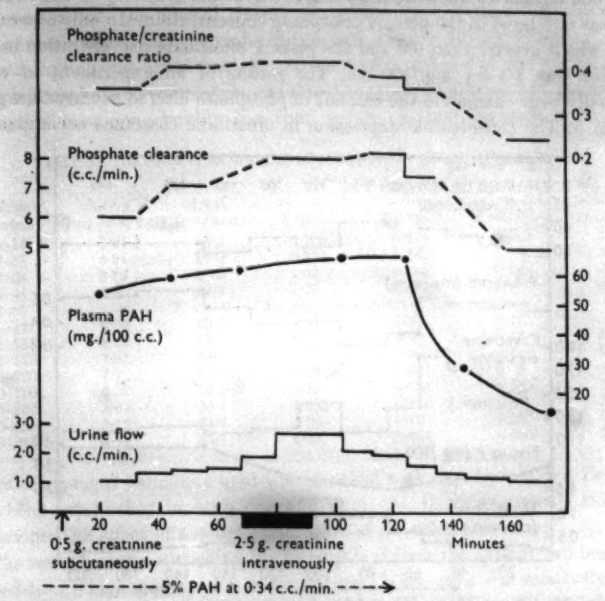

Fig. 4. Showing the reversible effect of a high concentration of PAH on phosphate clearance. Cat, 4·2 kg.

TABLE 1. The effect of plasma phosphate concentration on the amount of phosphate reabsorbed

	Plasma phosphate (mg. P/100 c.c.)		
	2–3	3–4	4–5
Normal	1·8 ±0·09 (20)	2·33±0·08 (28)	3·5±0·39 (26)
Thiosulphate	1·5 ±0·08 (4)	2·85±0·1 (13)	—
PAH>20 mg./100 c.c. plasma	1·21±0·11 (17)	1·98±0·07 (47)	3·5±0·57 (5)
PAH>100 mg./100 c.c. plasma	0·74±0·07 (4)	1·75±0·1 (14)	—

influence of thiosulphate (four experiments), and those under the influence of PAH (fifteen experiments). The difference between these latter values and the normal are highly significant, except in the small group at 4–5 mg. P/100 c.c. plasma. When values obtained at a plasma PAH concentration of over 100 mg./100 c.c. are compared, the difference is still more striking.

Even at low concentrations of plasma phosphate an appreciable amount was excreted in the urine, and it became apparent that the amount reabsorbed was a relatively constant fraction of that being filtered. This fraction varied from one animal to another, from 0·5 to 0·8 in the majority of experiments, and was unrelated to the plasma phosphate concentration. In only one experiment was it greater than 0·9 and the plasma phosphate concentration on this occasion was 4·3–5·4 mg./100 c.c. The results of an experiment in which unusually large changes in the amount of phosphate filtered occurred are given in Fig. 5. The considerable depression in creatinine clearance accompanying

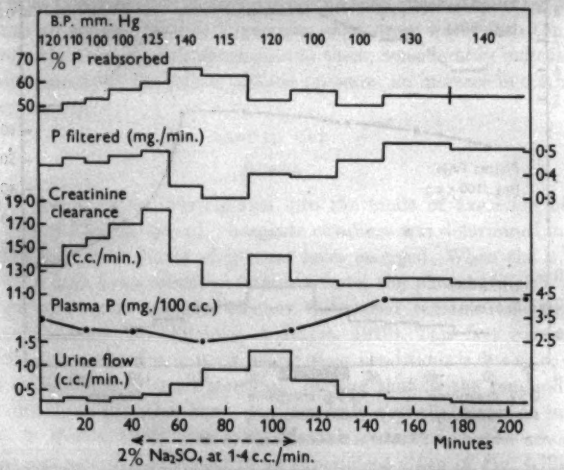

Fig. 5. Showing the relative constancy of the proportion of filtered phosphate which is reabsorbed and its independence of rate of urine flow or of the absolute amount filtered. Cat, 3·85 kg.

the onset of sulphate diuresis might have been due to an increase in intrarenal pressure, although the kidneys were quite flaccid at the end of the experiment. The amount of phosphate filtered varied from 0·32 to 0·53 mg. P/min., yet the percentage reabsorbed varied only from 49 to 66%, and it is clear from the figure that these variations were not systematically related to any of the other variables. A considerable part must, in fact, be attributed to experimental error, for the values are dependent on the accuracy of analysis of both phosphate and creatinine in both plasma and urine.

The absence of any *Tm* value for phosphate reabsorption over the range of concentration encountered led us to examine the effect of raising the concentration artificially by injection of sodium phosphate. Forty minutes after the end of the injection, the plasma phosphate concentration was 9·0 mg. P/100 c.c.,

and as it fell steadily to 5·5 mg. in the following 3 hr., the amount reabsorbed varied from 2·45 to 3·15 mg. P/100 c.c. G.F.R. These values were well below the average normal value at 4–5 mg. P/100 c.c. plasma and led us to repeat the experiment with, in addition, an initial determination of the normal value. By good fortune, the normal plasma phosphate concentration was one of the highest encountered and the reabsorption rate was 3·5–4·0 mg. P/100 c.c. G.F.R. When sampling was again made 40–60 min. after the end of the injection, the plasma phosphate concentration was raised to 8 mg. P/100 c.c., but the amount reabsorbed was considerably less than before, as may be seen in Table 2.

TABLE 2. The effect of an injection of phosphate on phosphate reabsorption

Time (min.)	Plasma P (mg./100 c.c.)	G.F.R. (c.c./min.)	P (mg./min.) Filtered	Excreted	Reabsorbed	mg. P reabsorbed/ 100 c.c. G.F.R.
0–13	5·01	15·9	0·798	0·235	0·563	3·55
13–23	4·91	19·0	0·935	0·252	0·683	3·6
23–34	5·02	20·3	1·02	0·282	0·738	3·65
34–44	5·35	23·3	1·25	0·293	0·957	4·1
47–65		70 c.c. 0·1 M-Na phosphate (pH 7·4) intravenously				
107–121	8·05	20·7	1·67	1·18	0·49	2·4
121–133	8·02	17·8	1·43	0·95	0·48	2·7
133–143	7·81	23·0	1·80	1·10	0·7	3·05
143–160	7·1	23·6	1·68	1·02	0·66	2·8
160–180	6·32	22·5	1·42	0·835	0·585	2·6
180–200	5·61	24·7	1·385	0·815	0·57	2·3
200–220	5·13	24·6	1·26	0·73	0·53	2·15
220–240	4·79	25·7	1·23	0·635	0·595	2·3

Since the general technique used was identical with that by which a *Tm* value has been established in both dog and man, this surprising result led us to determine the effect of a constant infusion of phosphate.

The results varied according to the length of time the animal had been subjected to a high concentration of phosphate. If the time was relatively short, the reabsorption rate was higher than that encountered normally. Maintenance of the raised concentration resulted in a reduction to normal or less than normal. The results of an experiment in which both effects were observed are given in Fig. 6. As the plasma phosphate concentration increased, the amount reabsorbed increased steadily, to reach a maximum value of 5 mg. P/100 c.c. G.F.R. at 35–50 min. after the beginning of the infusion, but thereafter it fell again in spite of a steadily increasing concentration in the plasma. In two further experiments of the same nature in which sampling was delayed until a fairly steady concentration in the plasma had been attained, no such peak was observed. A summary of the results of these two, together with that of a further experiment in which the early peak stage was mainly studied, are given in Table 3. One point of interest is common to both figure and table. When the infusion is stopped and the plasma phosphate concentration allowed to fall, the rate of phosphate reabsorption is greatly diminished below the normal value. In the figure, a final value of 1 mg. P/100 c.c. G.F.R. is to be seen in place of

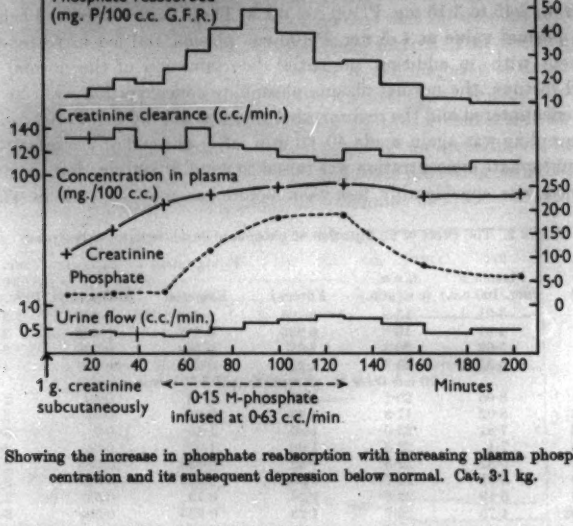

Fig. 6. Showing the increase in phosphate reabsorption with increasing plasma phosphate concentration and its subsequent depression below normal. Cat, 3·1 kg.

TABLE 3. The immediate and after-effects of an infusion of phosphate on phosphate reabsorption

Exp.	Time (min.)	Plasma P (mg./100 c.c.)	G.F.R. (c.c./min.)	mg. P reabsorbed/ 100 c.c. G.F.R.
1. Cat, 4·4 kg.	0–45	3·93–4·46	12·3–13·2	2·87±0·1 (4)
	45–99	0·1 M-Na phosphate (pH 7·4) intravenously at 0·63 c.c./min.		
	45–64	7·1	14·7	2·97
	64–99	9·7	17·8	3·44
	99–153	0·4 M-Na phosphate (pH 7·4) intravenously at 0·63 c.c./min.		
	111–123	22·8	16·9	5·8
	123–139	29·0	16·2	6·3
	139–153	32·2	15·65	5·0
2. Cat, 3·65 kg.	0–10	3·81	22·7	2·13
	10–19	3·77	24·5	2·34
	19–186	0·15 M-Na phosphate (pH 7·4) intravenously at 0·63 c.c./min.		
	45–186	7·49–9·91	20·1–25·0	3·16±0·12 (6)
	322–336	3·12	22·8	0·93
	336–350	2·87	21·2	0·9
3. Cat, 4·7 kg.	0–48	2·0–2·08	16·5–21·8	2·0 ±0·15 (4)
	49–202	0·21 M-Na phosphate (pH 7·4) intravenously at 0·4 c.c./min.		
	92–202	11·0–15·3	12·8–18·0	2·66±0·09 (6)
	322–340	4·48	12·6	1·95
	340–352	4·31	14·1	2·09

the normal 3·5 or more. In Exp. 2, in the table, a value of 0·9 is found in place of the expected 2·0 or more, and in Exp. 3 a value of 2·0 in place of the expected 3·5. It should be noted that a period of 2 hr. was allowed to elapse before the final sampling in these two experiments.

Discussion

The pattern of phosphate reabsorption in the cat is, in some respects, different from that in the dog. However low the plasma phosphate concentration may be, the urine is never found to be phosphate-free, the minimum value observed being 6% of that filtered. Within the limits of concentration encountered, 2–5 mg. P/100 c.c. plasma, both the amount reabsorbed and that excreted increase with increasing plasma phosphate concentration. Qualitatively the same effect is observed in the dog. Thus, Jahan & Pitts (1948) give figures showing a steady increase in excretion of phosphate, from 4 to 50% of that filtered, as the plasma phosphate concentration increased from 3 to 7 mg. P/100 c.c., during which time the amount reabsorbed had also steadily increased to reach a *Tm* value at 7 mg. P/100 c.c. plasma. In the cat, however, the amount reabsorbed appears to be a more or less constant fraction of that filtered. It varies somewhat from one animal to another but, with few exceptions, lies between 0·5 and 0·8. The higher fractions are not associated with low plasma phosphate concentrations, as might be expected; for example, a value of 0·65 was obtained at 2·2 mg. P/100 c.c. plasma in one experiment, while values of 0·9–0·95 were observed in another at 4·5–5·0 mg. P/100 c.c.

The response of the cat to an artificially raised plasma phosphate concentration is also very different from that of the dog or man. In these the amount of phosphate reabsorbed increases with increasing concentration to a certain point and then remains constant. In the dog a value of 3·5–4·0 mg. P/100 c.c. G.F.R. is attained (Pitts & Alexander, 1944), and in man a value of 3·63 has been established (Barclay *et al.* 1944). Should the value be constant from one species to another, the average value of 3·5 observed at a plasma phosphate concentration of 4–5 mg. P/100 c.c. would suggest that a similar point had been reached in the cat. Yet the higher values observed in the early stages of the infusion cast doubt on the idea. These cannot reasonably be attributed to technical errors for, although the plasma phosphate concentration was increasing fairly rapidly, the urine samples were relatively large and the plasma phosphate analysis was made on arterial blood. They may, nevertheless, be fictitiously high: if some of the injected phosphate is temporarily bound to plasma protein, the amount actually filtered, and thus the calculated amount reabsorbed, wil be less than that recorded. Dialysis of such plasma indicates that all the phosphate is free, but this provides no evidence regarding a possible temporary binding by protein.

At the moment, therefore, the question of the existence of a *Tm* value must remain an open one, owing to the unusual reaction of the cat to a prolonged increase in plasma phosphate concentration. Whether this be effected by constant infusion or by a single injection, the amount of phosphate reabsorbed is less than the maximal normally observed at naturally occurring concentrations of 4–5 mg. P/100 c.c. plasma; and when the concentration is later allowed to fall, the amount reabsorbed falls to abnormally low values. It seems possible, in fact, that the process of phosphate reabsorption in the cat may be fundamentally different from that in man and dog. The depression of reabsorption or secretion of substance with an established *Tm* value by a high concentration of such a substance, sometimes attributed to 'fatigue' of the responsible mechanism, is readily reversible when the concentration is allowed to fall. The long-continued depression in phosphate reabsorption in the cat is in no way comparable, and an understanding of its mode of action, as that of the reabsorptive mechanism itself, must await more detailed investigation. The fact that a constant proportion of the filtered phosphate is reabsorbed suggests that the reabsorption may, in fact, be passive, comparable with the obligatory reabsorption of water and other salts.

A further difference between the cat and the dog, which may however be apparent rather than real, is the relationship between the amount of phosphate reabsorbed and G.F.R. In dogs (Ayer, Schiess & Pitts, 1947) the absolute amount reabsorbed remains constant with widely varying filtration rates, whereas in cats the amount reabsorbed varies in the same direction as the G.F.R. In the dogs, however, the variation in G.F.R. was induced by protein feeding and can be ascribed to increase in filtration in nephrons already functioning. Under such conditions the amount of phosphate reabsorbed should not vary, provided the plasma phosphate concentration is such that maximum reabsorption is already occurring. In the cats, variation in G.F.R. in any one animal was normally due to circulatory changes owing to depth of anaesthesia, etc. and, although it is generally assumed that such variation is effected by a change in filtration rate at each glomerulus, it is possible that, instead, it is due to a complete closure of some nephrons with the remainder functioning fully. On the other hand, in view of the difference in mode of reabsorption in the two species, it is equally possible that all the nephrons are functioning all the time; since the amount of phosphate reabsorbed is a constant fraction of that filtered, any change in G.F.R. would have the same effect as a change in plasma phosphate concentration.

Before these facts concerning phosphate reabsorption were known, its behaviour under the influence of thiosulphate had been accepted as evidence that the toxic effect of this substance on the kidney was to put some nephrons completely out of action leaving the remainder intact. This interpretation is no longer valid, but the additional evidence, that the secretion of both thio-

sulphate and PAH are reduced to the same extent as is the filtration rate, still remains in support of the original hypothesis.

PAH, on the other hand, while depressing G.F.R. to a minor degree—and this is generally attributed to circulatory changes—has a greater depressive action on the tubules, seriously reducing the amount of phosphate reabsorbed. PAH is thought to be secreted in the distal tubule and phosphate reabsorbed in the proximal tubule; it is difficult to see how one process could affect the other. At high concentrations, however, it has been shown that PAH is also reabsorbed (Eggleton & Habib, 1950). The fact that the degree of this reabsorption varies with the concentration in the plasma and not with the U/P ratio suggests that if such reabsorption be passive, it would occur in the proximal tubule. The fact that it interferes with phosphate reabsorption is in favour of the suggestion that both processes may be active.

Since these experiments were completed, West & Rapoport (1949) have reported a similar effect in man. Phosphate excretion was considerably increased when PAH was injected, the magnitude of the increase depending on that of the plasma PAH concentration. These authors consider the competition, between phosphate reabsorption and PAH secretion, to be for a common source of energy of limited capacity.

SUMMARY

1. Phosphate excretion in man frequently varies with G.F.R. and with urine pH, but statistical analysis of the results indicates a significant correlation only with G.F.R. Neither urine pH nor rate of flow have any influence on phosphate output (Figs. 1 and 2).

2. The amount of phosphate reabsorbed in the kidney of the cat varies with plasma phosphate concentration under normal conditions, the amount remaining a fairly constant fraction of that presented to the tubules (Table 1 and Fig. 5).

3. On raising the plasma phosphate concentration artificially there is an immediate apparent increase in phosphate reabsorption, followed by a depression below normal which is maintained when the concentration is allowed to return to within the normal range. The existence of a Tm value is, therefore, uncertain (Fig. 6, Tables 2 and 3).

4. The amount of phosphate reabsorbed is depressed by a high concentration of PAH (Fig. 4 and Table 1).

Our thanks are due to Mr K. Spencer for help in performing some of the phosphate analyses in the cat experiments, to Dr Florence N. David of the Statistics Department, University College, London, for supplying us with a suitable statistical formula, and to Dr L. E. Bayliss for helpful suggestions in interpretation of the results.

REFERENCES

Ayer, J. L., Schiess, W. A. & Pitts, R. F. (1947). *Amer. J. Physiol.* **151**, 168.
Barclay, J. A., Bray, H. G. & Cooke, W. T. (1944). *J. Physiol.* **103**, 7*P*.
Dean, R. F. A. & McCance, R. A. (1948). *J. Physiol.* **107**, 182.
Eggleton, M. G. (1942). *J. Physiol.* **101**, 1*P*.
Eggleton, M. G. (1947). *J. Physiol.* **106**, 456.
Eggleton, M. G. (1949). *J. Physiol.* **108**, 482.
Eggleton, M. G. & Habib, Y. A. (1949). *J. Physiol.* **110**, 98.
Eggleton, M. G. & Habib, Y. A. (1950). *J. Physiol.* **110**, 458.
Haldane, J. B. S. (1921). *J. Physiol.* **55**, 265.
Harrison, H. E. & Harrison, H. C. (1941). *J. clin. Invest.* **20**, 47.
Havard, R. E. & Reay, G. A. (1926). *J. Physiol.* **61**, 35.
Hendrix, B. M. & Calvin, D. B. (1925). *J. biol. Chem.* **65**, 197.
Jahan, I. & Pitts, R. F. (1948). *Amer. J. Physiol.* **155**, 42.
McCance, R. A. & Young, W. F. (1941). *J. Physiol.* **99**, 265.
Ollayos, R. W. & Winkler, A. W. (1943). *J. clin. Invest.* **22**, 147.
Pitts, R. F. & Alexander, R. S. (1944). *Amer. J. Physiol.* **142**, 648.
West, C. D. & Rapoport, S. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 322.

J. Physiol. (1950) 111, 437–444 612.891.547.435.292

ACETYLCHOLINE SYNTHESIS IN NORMAL AND DENERVATED SYMPATHETIC GANGLIA OF THE CAT

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MacIntosh (1938) was the first to relate the time of failure of conduction through a denervated sympathetic ganglion with a diminution in its acetylcholine content. From the work of Brown & Feldberg (1936) on perfused ganglia, and of Feldberg (1943) on incubated extracts of ganglia, it appeared that both events might be attributed to a failure of the preganglionic endings to synthesize acetylcholine. However, Feldberg's methods (1943) were relatively insensitive (Nachmansohn, John & Berman, 1946) and did not provide unequivocal evidence that the reduced acetylcholine synthesis obtained in denervated ganglia was due to loss of the enzyme concerned (choline acetylase) rather than to loss of some other constituent of the system such as the activator fraction. We therefore decided to repeat Feldberg's work using the more refined methods he later developed (Feldberg & Mann, 1945, 1946). These methods provide a means of determining the choline acetylase activity of a tissue independently of other variables, and have been adapted in our experiments so that synthesis measurements could be made on individual sympathetic ganglia of the cat.

METHODS

Operative and experimental procedures. Twenty-nine cats and three kittens were used for the tests. Of these, nine animals with no previous operation were treated as controls; in seventeen the superior cervical ganglion on one side was denervated (aseptically) by the removal of 1–2 cm. of the cervical sympathetic trunk from a site low in the neck. In three others the vagus nerve central to the nodose ganglion and the post-ganglionic trunk of the superior cervical ganglion were both cut on the one side. The operations were performed 1–29 days before the acute experiments. For the latter, anaesthesia was obtained either by Pentobarbitone sodium, 40 mg./kg., or by 'Dial' (Ciba), 0·7 ml./kg., injected intraperitoneally or intravenously. After tracheotomy, the two cervical sympathetic nerves were prepared for stimulation. Voltages ranging from 0·5 to 20 V., with pulse durations of 0·1 or 1 msec. were delivered from a 'Ritchie-Sneath' Square-Wave Stimulator. The nerve was stimulated for 5 sec. at approximately fifty shocks per sec. and contractions of the nictitating membrane were recorded to provide evidence for ganglionic transmission. In the nodose ganglion experiments, the peripheral end of the vagus was tested for stretch afferent

activity by means of a suitable amplifier and loudspeaker. The extent of degeneration of the cardio-inhibitor fibres of the vagus was examined by stimulating the peripheral end of the trunk and noting the effect on the heart-rate.

Preparation of ganglion powders. Each ganglion was taken from the living cat and transferred to ice-cold saline for about 30 sec., and then removed to an ice-cold dark glass plate where it was dissected out of its connective tissue sheath and all branches trimmed off. Usually its wet weight was measured on a torsion balance. This procedure occupied 1–2 min. The ganglion was stored in a cooled tared 10 ml. centrifuge tube in the freezing compartment of a refrigerator until the other ganglia had been similarly prepared (35–50 min. according to the number of ganglia removed).

Each ganglion was dried in its test-tube by suspension in 5–8 ml. of ice-cold acetone, in which it was chopped rapidly with fine scissors into fragments. After 10–15 min. stirring, the suspension was centrifuged and the acetone decanted. The tubes containing the dried ganglion fragments were then stored in a vacuum desiccator in a refrigerator for 2½ hr., a period sufficiently long for them to attain constant weight. They were then weighed on an air-damped balance at room temperature. In estimating the weight of the tissue the probable error was within $\pm 0{\cdot}3$ mg.

Incubation and assay. To each tube containing the ganglion fragments the following reagents were added to make a total volume of 5 ml.: 2·9 ml. ice-cold 0·9% NaCl; 0·1 ml. each of 6·0% KCl, 3·0% choline Cl, 0·5% eserine SO_4, 4·0% $MgCl_2$, and 2·0% NaF; 1·0 ml. of 'activator extract' (equivalent to 50 mg. of acetone dried guinea-pig brain prepared by the method of Feldberg & Mann (1946)); 0·1 ml. of either 13·4 or 15·0% Na citrate; 0·1 ml. containing 4·5 mg. cysteine, and 0·4 ml. containing approx. 0·4 mg. ATP. pyroP (8 mg. of the original Ba salt). The tubes were then plugged with cotton-wool and incubated for 1 hr. in a water-bath at 40° C. The amount of acetylcholine which could be formed non-enzymatically was determined by incubating a control tube containing all of the above-named constituents save the dried ganglion tissue. The amounts so formed ranged from 0·00 to 0·15 μg. per tube.

After incubation, 1·0 ml. of 0·33 N-HCl was added to each tube, which was then heated to boiling-point. After being cooled and neutralized, its acetylcholine content was then assayed on the frog rectus abdominis muscle by the method of Chang & Gaddum (1933). All precautions outlined by Feldberg (1945), Feldberg & Mann (1945, 1946) and Feldberg & Hebb (1947) were taken to avoid any errors which might have arisen due to the presence of substances in the extracts which potentiate or simulate the muscle response to acetylcholine. It should be pointed out that while these substances may not be capable of initiating a contraction themselves, they may yet augment the response of the muscle to acetylcholine so, unless such precautions are taken, the yield of acetylcholine may be seriously overestimated.

This method of assay enabled us to estimate a minimum of 0·05 μg. acetylcholine per tube. Since the weight of a dried ganglion was of the order of 1–2 mg. this represented values of 50–100 μg./g. dried tissue. Yields falling below this figure could not therefore be reliably measured.

It should be mentioned that in the present experiments the amount of *added* activator fraction was kept constant in all tests. It was added in excess in order that the activator content of the ganglion should have no influence on synthesis. Thus, variations in the yield of acetylcholine were dependent upon the enzyme activity *per se*. In this respect the method differed from that advocated by Feldberg & Vogt (1948) for the estimation of choline acetylase activity in small samples of nerve tissue.

RESULTS

Acetylcholine synthesis in normal sympathetic ganglia

The quantity of acetylcholine synthesized by individual normal sympathetic ganglia *in vitro* was found to average from 7 to 8 μg./hr. The ranges of values found are given in Table 1, but the yields have not been expressed in mg./g. powder/hr., because there was not a constant relation between the amounts of acetylcholine synthesized and ganglion dry weights. (Average figures for

ganglion dry weights are given in Table 2.) This may be due to the fact that the dry-weight figures are not necessarily a measure of the amount of preganglionic tissue in a ganglion.

TABLE 1. Acetylcholine synthesis in normal ganglia

Ganglion	Mean Ach synthesis (μg./tube/hr.)	Range (μg./tube/hr.)	No. of observations	Standard deviation
R. sup. cervical	7·2	3·0–13·5	17	2·4
Right stellate	8·3	3·4–14·5	22	2·7
Left stellate	8·0	4·2–13·0	23	2·3

Note. Values for the left superior cervical ganglion are not included since there was an insufficient number of comparable observations.

TABLE 2. Dry weights of cat sympathetic ganglia

Ganglion	Mean dry weight in mg.	Range in mg.	No. of observations	Standard deviation
R. sup. cervical	2·6	1·6–41	17	0·80
L. sup. cervical	2·0	1·7–2·7	10	0·63
R. stellate	2·8	1·9–4·4	22	0·74
L. stellate	2·6	1·5–3·5	23	0·59
Denervated sup. cervical	2·3	1·5–4·2	18	0·74
Normal sup. cervical	2·4	1·6–4·1	26	0·65

It may be seen from Table 1 that the acetylcholine yields of ganglia from different cats have a wide range (coefficient of variation 33%); however, the differences in yield between ganglion pairs from any one animal were not quite so large (coefficient of variation: stellate ganglia 22·5%, superior cervical ganglia 16·7%). The total variation could only partly be ascribed to the method of incubation and assay, since aliquot samples treated identically and incubated together showed a coefficient of variation of 12·5%. The activator extracts used were not equally efficient, but neither did this completely account for the variations.

The choline acetylase activity of normal sympathetic ganglia, when calculated as mg. acetylcholine synthesized/g. powder/hr., was high compared with that of other nervous tissue. The maximum figures, for mammalian tissue, published by other workers using comparable methods, are 1·6–2·4 mg./g. powder/hr. (Nachmansohn & Berman (1946) rabbit brain), and 1·5–1·8 mg./g. powder/hr. (Feldberg & Mann (1946) guinea-pig brain). In our experiments guinea-pig brain powders, tested prior to their use as a source of activator under conditions strictly comparable with the ganglion experiments, gave lower values, ranging from 0·8 to 1·6 mg./g. powder/hr. On the other hand, the average acetylcholine yield from sympathetic ganglia was in the region of 3 mg./g. powder/hr.; 4 mg./g. powder/hr. or more was obtained from twenty-eight of the eighty-seven normal ganglia tested; while the maximum yield was 6·5 mg./g. powder/hr. The highest values were obtained from the sympathetic ganglia of young kittens from 9 to 10 weeks old, as may be seen from the data given in Table 3.

Coeliac ganglia also synthesize acetylcholine. This was found in three experiments on adult cats. The acetylcholine yield ranged from 3·0 to 5·5 μg./ganglion/hr. The dry weights of ganglion pairs, however, were very unequal. The weight of the left in terms of the right varied from 50 to 300%. This was due to the difficulty in defining the precise limits of the ganglia for removal; their structure appeared to be very variable.

TABLE 3. A comparison of acetylcholine synthesis in cat and kitten sympathetic ganglia

Group	Cat weight in kg.	μg. Ach/ganglion range	Average μg. Ach/ganglion	Ganglion dry weight range	Average ganglion dry weight	μg. Ach/g. powder/hr. range	Average μg. Ach/g. powder/hr.
I (activator preparation 24)	0·57*	4·2–7·6	5·8	0·9–1·4	1·1	4740–6250	5500
	1·5*	6·0–9·5	7·4	1·0–1·6	1·4	4290–6000	5390
	2·5	6·5–8·8	7·8	1·8–2·2	2·1	3250–4000	3630
	3·1	7·2–8·8	7·5	1·8–2·2	2·0	3450–4200	3803
II (activator preparation 25)	0·62*	3·4–5·2	4·0	0·8–1·1	1·0	3270–6500	4530
	2·4	3·4–4·2	3·9	1·5–2·7	2·0	1700–2460	2130
	2·8	3·8–5·6	4·8	1·6–2·1	1·9	2240–2690	2520

*Figures for kitten experiments.

Nachmansohn, Hestrin & Voripaieff (1949) have reported that a protein fraction of the rabbit brain may synthesize a substance which is not chemically identifiable as acetylcholine but has a similar action on the frog rectus abdominis, the cat's blood pressure and the isolated frog heart (Middleton & Middleton, 1949). This substance is therefore indistinguishable from acetylcholine on such pharmacological tests. Since it was possible that this unknown substance might form part of the apparent acetylcholine yield obtained in our experiments some further observations were made to test this point. According to Nachmansohn, the newly discovered acetylcholine-like compound is synthesized in the absence of choline. We therefore compared the yields obtained on incubating pairs of stellate and superior cervical ganglia, the one side with, the other side without, the addition of choline. Our results showed that while the four ganglia incubated with choline formed the normal amounts of acetylcholine, see Table 1, those without choline formed hardly any at all, or 98–100% less than the control (values were 0·4, 0·3, 0·0 and 0·0 μg. acetylcholine/ganglion/hr.). Almost all the active substance produced by normal ganglia must therefore have been acetylcholine. These observations are supported by other experiments undertaken in this laboratory (Hebb, unpublished, 1949) in which comparable tests on acetone dried guinea-pig brain powder were carried out. In these it was found that the amount of acetylcholine produced in the absence of added choline did not exceed 5% of the yield obtained in the presence of choline.

Acetylcholine synthesis in denervated superior cervical ganglia

The results obtained are graphically represented in Fig. 1. Choline acetylase activity of the test ganglion was significantly less than that of the control side when the period of denervation was 2 days or longer. By the fourth day it was only about 15% of the control side and in 4 weeks dropped to 2%. These results confirm the observations of Feldberg (1943) already mentioned. The time course

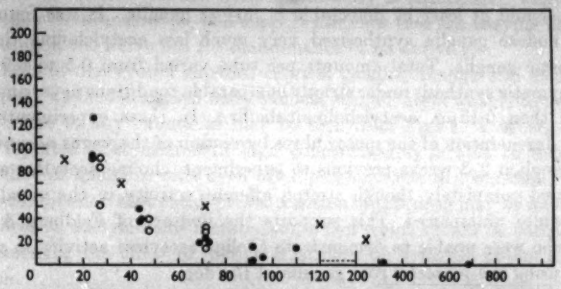


Fig. 1. Rate of disappearance of cholinergic enzymatic activity and acetylcholine from the denervated superior cervical ganglion of the cat. *Ordinate:* activity of denervated ganglion in percentage of activity of the control gland. ●, choline acetylase activity estimated from the synthesis of acetylcholine by incubated ganglia. ×, 'true' cholinesterase activity, figures from Sawyer & Hollinshead (1945). ○, acetylcholine content in percentage of the content of normal ganglion, figures from MacIntosh (1938). *Abscissa:* hours after section of the cervical sympathetic trunk.

of the loss of choline acetylase activity was similar to that for the fall in acetylcholine content of the ganglion as reported by MacIntosh (1938) and Feldberg (1943). This may be seen from Fig. 1, where MacIntosh's figures for acetylcholine content and Sawyer & Hollinshead's figures for true cholinesterase activity have also been plotted. 'True' cholinesterase activity evidently falls rather more slowly than does the activity of choline acetylase. In three experiments on animals, in which post-ganglionic section had been carried out 2–3 weeks earlier, the ganglia synthesized the usual amounts of acetylcholine.

In the denervated ganglion, failure in transmission occurred when its ability to synthesize acetylcholine was markedly reduced. At about 20 hr. after denervation the contraction of the nictitating membrane in response to electrical stimulation of the pre-ganglionic trunk appeared to be quite normal. After about 40 hr. the results were variable, but at 70 hr. the membrane response was hardly detectable or not present. These observations on transmission seem to agree with those of MacIntosh (1938) and other workers.

Acetylcholine synthesis in normal and denervated nodose ganglia

In view of the fact that MacIntosh (1941) found that nodose ganglia contained acetylcholine, tests on their ability to synthesize this ester were included in six experiments. The weights of the nodose ganglia when dried were found to range from 0·6 to 1·7 mg. Since the experiments on kitten ganglia had already shown that comparable amounts of tissue were capable of synthesizing large quantities of acetylcholine (Table 3) we felt that choline acetylase activity, if present, should at least be detectable in nodose ganglia. It was found that normal nodose ganglia synthesized very much less acetylcholine than the sympathetic ganglia. Total amounts per tube varied from 0·5 to 0·9 μg./hr. (non-enzymatic synthesis under strictly comparable conditions never amounted to more than 0·15 μg. acetylcholine/tube/hr.). In three experiments, after securing degeneration of the motor fibres by section of the vagus nerve central to the ganglion 2–3 weeks previous to experiment, choline acetylase activity disappeared completely though stretch afferent activity in the vagal trunk seemed quite unimpaired. This supports the findings of Feldberg & Mann (1946), who were unable to demonstrate choline acetylase activity in sensory ganglia, using the posterior root ganglia of the dog.

DISCUSSION

The experiments described in this paper repeat and extend the earlier published work by Feldberg, and our results confirm his in all essentials. It is clear that there is a significant loss of choline acetylase from denervated superior cervical ganglia as early as 40 hr. after preganglionic nerve section and that the loss is progressive, with a time course which is similar to that for the diminution in acetylcholine content. How much more sensitive the method is than that used by Feldberg in his earlier study (1943) is emphasized by the difference in yield of normal ganglia. He found that sympathetic ganglia synthesized about 0·07 mg./g. ganglion/hr. (recalculated from Feldberg's data on the assumption that the dry weight of tissue after acetone treatment is 20% of its wet weight), while our results show that individual ganglia synthesize about 3·0 mg./g. powder/hr. Our figures also place in perspective the very small amounts of acetylcholine synthesized by non-nervous tissue. Sympathetic ganglia are, of course, relatively simple units of the nervous system and probably contain a higher proportion of cholinergic endings than the less easily defined neurone groups of the brain and spinal cord, so that a higher rate of synthesis might be expected.

It might also be argued that the high rates of synthesis observed are due to the fact that the amount of 'activator' added was out of all proportion to the weight of the ganglion. This discrepancy may be more apparent than real, for it is possible that substances which diffuse out of cells after the death of a tissue

are yet fixed within them during its life. An example of such a substance is carnosine (Eggleton & Eggleton, 1933). The same may be true for the activator: *in vivo* it may be very concentrated locally in relation to choline acetylase, a relationship which acetone treatment would terminate. Degenerative changes in the ganglion might also alter the localization of activator relative to enzyme, and the failure of synthesis revealed by previous investigators could have been attributed to loss of this fraction. Our experiments on denervated ganglia show clearly, however, that choline acetylase activity is diminished, whatever the fate of the activator may be, and support the view that the enzyme ultimately depends upon the nerve cell for maintenance of its activity.

The cells of denervated superior cervical ganglia, though still excitable to the directly applied electrical and chemical stimuli, seem unable to synthesize acetylcholine to any extent, as may be seen from Fig. 1. Feldberg & Hebb (1948) reported that eserine in high concentrations is able to depress the excitability of ganglion cells even after preganglionic degeneration, which is a further indication that the depressant action of this drug may be independent of its effect on acetylcholine metabolism, a fact which should be borne in mind when assessing the effect of eserine treatment on synaptic potentials in the ganglion.

That superior cervical ganglion cells themselves do not synthesize acetylcholine to any extent is also interesting in the light of some of de Castro's work (1942). He reported that he had been able, on occasions, to establish a functional union between the sensory components of the vagus and the cells of the denervated superior cervical ganglion. Our results on the nodose ganglia indicate that, for periods up to 3 weeks after their separation from the brain, the sensory fibres of the vagus nerve might be acetylcholine free. Both observations may be considered to favour the view that the functional union obtained by de Castro did not involve cholinergic components.

Finally, our results imply that superior cervical ganglion cells and vagal sensory ganglion cells are unable to synthesize acetylcholine to any extent while capable of their expected responses. This evidence, in our view, militates against the theory that acetylcholine acts as a universal transmitter in the nervous system.

SUMMARY

1. A method is described for measuring acetylcholine synthesis in individual nervous ganglia of the cat. Superior cervical, stellate, coeliac and nodose ganglia were tested for choline acetylase activity by this method.

2. The highest figures for acetylcholine synthesis were obtained from normal superior cervical and stellate ganglia. These averaged about 8 μg. acetylcholine/ganglion/hr., and expressed conventionally this figure represents 3 mg. acetylcholine/g. acetone-dried powder/hr. In 30% of ganglia 4 mg. acetylcholine/g. powder/hr. or more was obtained.

3. The synthesis of acetylcholine by denervated ganglia was also measured. About 40 hr. after denervation of a ganglion, at a time when impulse transmission was usually impaired, its choline acetylase activity was only about 40% of the value for the control side. About 90 hr. after denervation synthesis was less than 20% of the normal side.

We wish to thank Miss C. O. Hebb for her advice and criticism throughout the experiments, and in the writing of the paper. We would also like to acknowledge the help given by Miss Hebb, Mr W. E. Balfour and Dr Tabrich in making some of the ATP., and the technical assistance given by Mr T. A. Black and Miss M. Forfar. Finally we are indebted to the Medical Research Council for a personal grant (R. J. B.) and to the Agricultural Research Council for a training grant (M. S.)

REFERENCES

Brown, G. L. & Feldberg, W. (1936). *J. Physiol.* **86**, 290.
Castro, F. de (1942). *Trab. Lab. Invest. biol. Univ. Madr.* **34**, 217.
Chang, H. C. & Gaddum, J. H. (1933). *J. Physiol.* **79**, 255.
Eggleton, P. & Eggleton, M. G. (1933). *Quart. J. exp. Physiol.* **23**, 391.
Feldberg, W. (1943). *J. Physiol.* **101**, 432.
Feldberg, W. (1945). *J. Physiol.* **103**, 367.
Feldberg, W. & Hebb, C. O. (1947). *J. Physiol.* **106**, 8.
Feldberg, W. & Hebb, C. O. (1948). *J. Physiol.* **107**, 210.
Feldberg, W. & Mann, T. (1945). *J. Physiol.* **104**, 8.
Feldberg, W. & Mann, T. (1946). *J. Physiol.* **104**, 411.
Feldberg, W. & Vogt, M. (1948). *J. Physiol.* **107**, 372.
MacIntosh, F. C. (1938). *Arch. int. Physiol.* **47**, 321.
MacIntosh, F. C. (1941). *J. Physiol.* **99**, 436.
Middleton, S. & Middleton, H. H. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 523.
Nachmansohn, D. & Berman, M. (1946). *J. biol. Chem.* **165**, 551.
Nachmansohn, D., Hestrin, S. & Voripaieff, H. (1949). *J. biol. Chem.* **180**, 875.
Nachmansohn, D., John, H. M. & Berman, M. (1946). *J. biol. Chem.* **163**, 475.
Sawyer, C. H. & Hollinshead, W. H. (1945). *J. Neurophysiol.* **8**, 137.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY

23 *April* 1949

Oscillations of the human iris. By F. W. Campbell and T. C. D. Whiteside. *Institute of Physiology, University of Glasgow*

Sustained oscillations of the human iris can be readily induced by focusing a narrow slit of light on the pupil margin with a clinical slit-lamp (Stern, 1944). The oscillations have been studied by direct observation and by cinematography using ultra-violet light.

In a group of sixty normal subjects the mean frequency was 69 oscillations/min. and the standard deviation was 11 oscillations/min. This frequency is higher than that of the spontaneously occurring oscillations encountered in normal and abnormal subjects.

The frequency is temporarily raised after dark adaptation, and lowered after light adaptation and after exercise. These changes in frequency may be accounted for by alteration in pupil size.

Thirty-seven healthy subjects were examined, and a significant positive correlation was found between the resting pulse-rate and the oscillation frequency of the iris.

REFERENCE

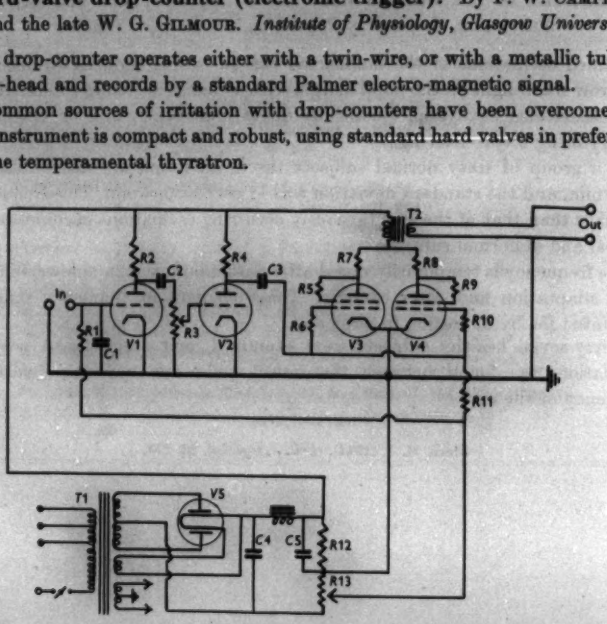
Stern, H. J. (1944). *Brit. J. Ophthal.* **28**, 275.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY
30 *July* 1949

Hard-valve drop-counter (electronic trigger). By F. W. Campbell and the late W. G. Gilmour. *Institute of Physiology, Glasgow University*

This drop-counter operates either with a twin-wire, or with a metallic tubular drop-head and records by a standard Palmer electro-magnetic signal.

Common sources of irritation with drop-counters have been overcome, yet the instrument is compact and robust, using standard hard valves in preference to the temperamental thyratron.



The circuit responds to the sudden decrease in resistance across the drop-head, and then the electromagnetic signal returns to its initial position even if the drop remains across the contacts. The following drop, causing a still further decrease in resistance, again activates the circuit. A drop with a resistance as high as half a megohm or as low as 500 Ω. will trip the circuit even when the previous drop adheres between the contacts. The instrument can be used with tap water or saturated sodium chloride solution.

When an accumulation of fluid between the contacts falls off there is no response, since the circuit responds only to a decrease in resistance. Operation is on 50 cyc./sec. a.c. independent of mains fluctuations from 190 to 260 V., and no other power source is required.

COMPONENTS LIST

R1, 0·25 MΩ., carbon, ½ W.
R2, 0·15 MΩ., carbon, 1 W.
R3, 0·5 MΩ., carbon, potentiometer. S./L.
R4, 0·15 MΩ., carbon, 1 W.
R5, 100 Ω., carbon, 1 W.
R6, 10 KΩ., carbon, ½ W.
R7, 100 Ω., carbon, 1 W.
R8, 100 Ω., carbon, 1 W.
R9, 100 Ω., carbon, 1 W.
R10, 10 KΩ., carbon, ½ W.
R11, 0·25 MΩ., carbon, ½ W.
R12, 33 KΩ., wire-wound, 10 W.
R13, 5 KΩ., wire wound, potentiometer, 5 W. (pre-set).
C1, 0·1 μF., paper.
C2, 0·005 μF., mica.
C3, 0·1 μF., paper.
C4, 8·0 μF., electrolytic, 500 V. working.
C5, 16·0 μF.
V1 and V2, 6SN7 *gt.*
V3 and V4, 6L6.
V5, 5Z4 *g.*

Smoothing choke: High-grade 'Radio' component, d.c. resistance not greater than 200 Ω.

Mains transformer: Primary as required. Secondaries: no. 1, 700 V. r.m.s., C./T., 150 mA. (h.t.); no. 2, 5 V. at 2 A. (rectifier heater); no. 3, 6·3 V. at 5 A. (valve heaters).

Output transformer: This component needs to be carefully chosen. General requirements are a primary d.c. resistance not greater than 100 Ω., high primary inductance, low leakage inductance, generous core-area and high primary-secondary-core insulation. It is important to bear in mind that this transformer is concerned only with pulse-transfer without respect to wave-form, therefore the ratio must be chosen to match the a.c. resistance of the output valves to that of the output-marker. The a.c. resistance of the output pair in this case is in the region of 300 Ω., and if the Palmer B17 marker were used (resistance 3 Ω.) this would imply a step-down ratio of 10–1. The Ferranti OPM5 was found to be particularly suitable; using the 10–1 ratio no marked variation in power-output was experienced with various types of electro-magnetic counters of resistance varying from about 1 to 30 Ω.

Case: 'Eddystone', cat. no. 644.

DESCRIPTION OF CIRCUIT FUNCTION

V1 is biased negatively to the cut-off region by the voltage-drop across R13, and therefore can accept only positive-going signals. When a drop of semi-conducting liquid 'makes' the input circuit the resultant reduction in negative bias is seen by the valve as a positive-going pulse, and this pulse appears in the anode circuit as an amplified negative-going pulse. This pulse is applied to the grid of V2 via the differentiator C2–R3 (time-constant 2·5 m.sec.), appearing at the anode as a sharp positive-going spike, of amplitude sufficient to overcome

the standing negative bias applied to the output valves. This pulse is applied to the output grids via C3 and R11, causing the circuit to trigger.

Should a drop of liquid adhere between the input terminals, provided the drop-resistance be not less than 500 Ω. the circuit will continue to be triggered by subsequent drops, there being still enough negative bias applied to V1 to enable a negative-going pulse to appear on its anode. R1 prevents short-circuiting of the bias system, whilst R1–C1 acts as a smoothing and decoupling filter for V1 grid, preventing a.c. interference. Should the adhering drop fall and thus open the input circuit, there will be no response, since this is equivalent to a negative-going signal on the input grid.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY
5 *November* 1949

Electromyographic study of standing in man. By W. F. Floyd and P. H. S. Silver. *Department of Physiology and Department of Anatomy, Middlesex Hospital Medical School, London, W.* 1

Surface electrodes were sited over the abdominal muscles and erectores spinae, and action potentials recorded simultaneously with the Ediswan standard six-channel electroencephalograph. In the erect position action potentials recorded from recti abdominis and erectores spinae are minimal or zero, even at high amplification. It would seem that in man the disposition of skeleton and soft tissues is such that the unstable erect position can be maintained with minimal muscle action. Surface electrode recording does not, however, exclude the possibility of slight activity, in the deeper fibres.

Trunk flexion is initiated by a short burst of activity in recti abdominis, and is maintained by the erectores spinae acting against gravity with the recti relaxed. Similarly trunk extension is initiated by a short burst of activity in erectores spinae and is maintained by the recti acting against gravity, with the erectores relaxed. Other movements were also studied.

Observations suggesting an inhibitory effect of adrenaline on sympathetic vasoconstrictor impulses in man. By H. J. C. Swan. *Sherrington School of Physiology, St Thomas's Hospital, London, S.E.* 1

The action of adrenaline on the blood flow through the hand has been investigated by the venous occlusion plethysmograph. There was a decrease in the rate of blood flow during the infusion in all the following procedures, but after the infusion was discontinued, return of blood flow to the normal value was found to differ as follows:

A. (1) After intra-arterial infusions in normal subjects the flow returned to the basal value but did not exceed it. (2) After intravenous infusions in the sympathectomized, a similar response was observed.

B. After intravenous infusions in normal subjects the rate of flow increases above the resting level often to several times its basal value, and subsequently returns to its resting level.

These observations strongly suggest that the effect observed in B is due to inhibition of vasoconstrictor tonic impulses, possibly in the central nervous system or in the sympathetic ganglia.

The relation of blood pressure and stroke volume of the heart in healthy young men at rest. By J. M. TANNER. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London, S.E.* 1

Various equations relating stroke volume to some function of blood pressure in man have been proposed, but inadequately tested. Those of Liljestrand & Zander (1928) and Remington, Noback, Hamilton & Gold (1948) were tried against measured stroke volumes of fifty young men at rest. A high-frequency ballistocardiograph, the modified formula for stroke volume (Tanner, 1949*a*), and blood pressures taken by the Korotkoff auscultatory method were used.

There was no significant correlation between stroke volume and systolic, diastolic, pulse pressure, or the calculation from Liljestrand-Zander's formula; that from Remington's formula correlated 0·31, only barely significantly.

A lack of relation between these variables in a population of individuals all at rest does not permit deductions about relations in a single individual under varying physiological circumstances, and vice versa.

Liljestrand's formula was designed for the latter circumstances, but some authors have mixed these two sources of variability; the high correlation claimed by Remington seems partly due to this, partly to spurious correlation produced by using per-surface area ratios (Tanner, 1949*b*).

REFERENCES

Liljestrand, G. & Zander, E. (1928). *Z. ges. exp. Med.* **59**, 105.
Remington, J. W., Noback, C. R., Hamilton, W. F. & Gold, J. J. (1948). *Amer. J. Physiol.* **153**, 298.
Tanner, J. M. (1949*a*). *J. Clin. Invest.* **28**, 567.
Tanner, J. M. (1949*b*). *J. appl. Physiol.* **2**, 1.

Cortico-spinal excitation of a spinal motoneurone pool. By E. G. T. LIDDELL and C. G. PHILLIPS. *University Laboratory of Physiology, Oxford*

We have made electrical and myographic records of near-threshold contractions (M. tibialis anterior—cat) evoked by weak stimulation of the main motor cortex (clamped stigmatic cathode; square *current* pulses of measured strength, duration and frequency). Under barbiturates such responses remain constant for hours. They are abolished by pyramid section.

Single long pulses (20–30 msec. duration) yield single twitches, and, if repeated, rhythmic myograms. Sample motor units follow up to 15 cyc./sec.

With 5–10 msec. pulses, sample units tend to fire at submultiples of the stimulating frequency. Nevertheless the total myographic rhythm may follow up to 45 cyc./sec.

1 msec. pulses, below 50 cyc./sec., are without effect. If above 350 cyc./sec., movement is prompt; with intermediate frequencies, delayed and recruiting. Such rhythms are not expressed in the response. Pyramidal stimulation yields similar results.

Our findings harmonize with those of Adrian & Moruzzi (1939).

REFERENCE

Adrian, E. D. & Moruzzi, G. (1939). *J. Physiol.* **97**, 153.

The response of isolated cerebral cortex to a weak stimulus. By B. Delisle Burns. *National Institute for Medical Research, London, N.W.* 3

The preparation of a neurologically isolated slab of cerebral cortex in anaesthetized cats has already been described (Burns, 1949). Such preparations are electrically inactive unless stimulated. A weak electrical stimulus applied to the surface causes a local surface negativity, the greater part of which is restricted to an area within 2 mm. of the excited point (see also Adrian, 1936). With increase of stimulus strength the negative response increases until a maximal response occurs at about 5 times threshold strength. The distribution of potential across the cortical surface can be explained by assuming that the stimulus has excited some of a system of nerve processes, which pass in all directions beneath the stimulated point. The excitation wave spreads radially outward along them with a velocity of 2 m./sec., while these conductors appear to stretch approximately 1 cm. from end to end and to be distributed at random within a superficial cortical plane.

REFERENCES

Adrian, E. D. (1936). *J. Physiol.* **88**, 127.
Burns, B. Delisle (1949). *J. Physiol.* **110**, 9*P*.

The hypothermic action of phenergan in the rat. By D. R. Wood.*
Department of Pharmacology, University of Sheffield

Phenergan and some other antihistamine substances cause a fall in the rat's rectal temperature (Halpern & Briot, 1949). The effect is greater after adrenalectomy. The phenothiazine derivative, 3300 R.P., chemically similar to phenergan but without antihistamine activity, has no such effect.

* George Herbert Hunt Scholar, 1949, and with a grant from the Medical Research Fund, University of Sheffield.

It has been shown that the fall in rectal temperature produced by phenergan in the rat is accompanied, usually after an initial transient increase, by a considerable fall in the animal's O_2 consumption, measured by the method of Bargeton & Krumm-Heller (1949). The hypothermic action is considered to be largely due to a central action.

3300 R.P. had no effect on O_2 consumption but neoantergan and larger doses of phenergan often caused motor excitement and increased O_2 consumption, although the rectal temperature usually fell.

REFERENCES

Bargeton, D. & Krumm-Heller, C. (1949). *J. Physiol. Path. gén.* **41**, 119.
Halpern, B. N. & Briot, M. (1949). *C.R. Soc. Biol., Paris*, **144**, 633.

Restoration of normal lymph histaminase in adrenalectomized cats by adrenocortical extract. By A. CARLSTEN and D. R. WOOD.*
Department of Physiology, University of Lund, Sweden

Carlsten, Kahlson & Wicksell (1949) found a high histaminolytic activity in canine thoracic duct lymph. Carlsten (1949) has observed this in cats too and also finds that after adrenalectomy the activity increases more than 10-fold within 1 hr. and lasts for at least 8 hr.

We have confirmed this and have shown that this effect of adrenalectomy was not influenced by infusion of adrenaline but was reversed or prevented by infusion of adrenocortical extract in 5 of 10 cats.

* George Herbert Hunt Scholar, 1949, and with a grant from the Medical Research Fund, University of Sheffield.

REFERENCES

Carlsten, A. (1949). *Acta Physiol. Scand.* (in the Press).
Carlsten, A., Kahlson, G. & Wicksell, F. (1949). *Acta Physiol. Scand.* **17**, 370.

Haemogramm, serum protein and plasma volume in healthy, well-nourished East Africans. By H. LEHMANN and A. H. MILNE

The 'precoctic' blood picture as the explanation of macrocytic anaemia in Central Africa. By H. LEHMANN

The effect of inflation of an arterial occlusion cuff at the ankle on the blood inflow to the human calf. By A. D. M. GREENFIELD, R. L. MCCORRY and R. F. WHELAN

Postural fainting reactions in man. By W. BRIGDEN, SHEILA HOWARTH and E. P. SHARPEY-SCHAFER

Variations in the distribution of radioactive phosphorus produced by activity in peripheral nerves. By G. CAUSEY and G. A. WERNER

PROCEEDINGS

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A heat-flow meter. By H. Stafford Hatfield.
Imperial College, London

The device demonstrated measures directly the strength in thermal units per unit area per unit time of a current of heat passing through it. It consists of a thin disk (12 mm. diameter, 1·5 mm. thick) of an alloy of tellurium, coated on its two sides with fine copper gauze. The coatings are connected by fine copper wires to a portable mirror galvanometer. When the disk is placed in a current of heat at right angles to its direction of flow, a small difference of temperature is set up between the two sides of the disk, proportional to the heat flow and the thickness of the disk, and inversely proportional to its thermal conductivity. This difference of temperature between the two copper-tellurium junctions in the galvanometer circuit causes a thermoelectric current in the latter. The thermoelectric power of the tellurium alloy is some ten times greater, and its thermal conductivity some five times less, than that of the usual thermoelectric materials. It is thus possible for a sufficient sensitivity, in terms of microvolts per unit flow of heat, to be obtained with a thin disk of this material.

A microvolt, registering about 1 mm. on the scale of available portable galvanometers, is obtained by about 1·35 kg.cal./m.^{2}/hr. (0·5 B.TH.U./ft.^{2}/hr.). This corresponds to 0·002° C. difference in temperature between the two sides of the disk. From this we can estimate the probable effect of the interposition of the disk upon the heat flow measured. If, for example, this is 40 kg.cal./m.^{2}/hr. from the skin of a person in bed in a room at 17°, a potential of about 30 μV. is recorded, corresponding to a temperature drop across the disk of 0·06°, which is negligible in comparison with the total temperature difference of 20° between body and air. Readings on the forehead are usually about three times this. Experiments with inorganic systems have verified that the flow of heat through the disk is equal to that through its immediate surroundings within 2%, even when it is placed upon a surface losing or gaining heat by both radiation and convection. Obviously, however, it does not take account of heat loss by evaporation.

The disks are individually calibrated. The constant varies by less than 5% over the range from room temperature to 200° C. and is not altered by heating to 120° C. for several weeks. It is unfortunately not yet possible to attain perfect uniformity in manufacture, so every disk requires separate calibration. The internal resistance of disk and leads is 0·8 ohm, which has to be allowed for in reading with a low-resistance galvanometer. In all uses so far the disks have been fixed to surfaces by thin adhesive plastic tape. The fine copper leads attached to the disk are 11 in. long, leading to a terminal piece strong enough to carry ordinary twin flex for connexion to the galvanometer. The terminal piece, which may be a solid moulded block, or a soft rubber piece provided with tapes, is suitably secured so that a pull on the galvanometer connexion will not be transmitted to the disk.

Physiologists might find this device useful in one or two ways. The weighted mean of readings on suitably chosen parts of the body of resting persons clothed to be in the vasomotor control range might be a fair indication of basal metabolic rate. Such experiments as have been done on normal subjects have given results of the correct order, but a procedure suitable for use in ordinary room temperature would need working out. Another application might be to the indication of the blood supply to skin or other tissue. If one side of the disk is in contact with skin while the other side is exposed to an agency abstracting or supplying heat, such as the air, a qualitative indication is obtained of change in blood supply to the skin. This was tested by attaching a disk to the ear of a rabbit and recording changes in heat output associated with the vasodilation or vasoconstriction due to pentamethonium iodide or adrenaline respectively.

The effect of stimulation on the exchange of radioactive phosphorus in peripheral nerve. By F. Grande and D. Richter. *Neuropsychiatric Research Centre, Cardiff*

When the sciatic nerve of a frog which was previously injected with radioactive phosphate is suspended in Ringer solution containing 0·1% glucose and oxygenated with $O_2 + 5\% CO_2$, there is a slow loss of phosphorus from the nerve; this loss is not greatly affected by stimulation of the nerve or by the addition of sodium glutamate to the Ringer solution.

Phosphate ions can also pass from the Ringer solution into the nerve, and the rate of uptake is greatly increased by stimulation. In a series of eleven experiments the mean uptake of stimulated nerves exceeded that of control nerves by approximately 200%. Since kations are likely to be taken in with the phosphate ions, this process might be of physiological significance as a mechanism for maintaining the normal potassium content of the nerve.

Reflexly discharged sympathin in the cat. By J. D. P. Graham. *Pharmacology Department, Welsh National School of Medicine, Cardiff*

In non-pregnant cats with chronically denervated nictitating membrane, under chloralose, the inferior mesenteric and hypogastric ganglia, ovarian and broad ligament connexions, viscera and adrenal glands were removed by cautery. The ovarian vessels were cleaned and preserved. Cocaine 8 mg./kg. was given. Stimulation of the hepatic nerve or lumbar sympathetic chains raised the blood pressure and contracted the nictitating membrane; uterus was not affected. Compression of carotid arteries caused reflex rise in pressure, slight relaxation of nictitating membrane from failure of blood supply and no effect on uterus. Amounts of L-adrenaline which produced the same rise of pressure as the reflex pressor response relaxed the uterus and contracted the nictitating membrane strongly; equipressor amounts of L-noradrenaline did not affect the uterus and had a lesser effect on the nictitating membrane. Sympathin released by carotid reflexes in the cat resembles noradrenaline in effect.

The rhythmical activity of the rat diaphragm. By J. E. Hall, J. A. C. Knox, R. J. S. McDowall and M. J. Morley. *Physiology Department, King's College, University of London*

If the rat diaphragm in a bath of Krebs's solution (0·035% KCl) is stimulated with short square-wave stimuli which stimulate the nerve only, at a rate of 2 per sec., rhythmical variations in contraction height frequently appear which are not present if the rate of stimulation is decreased or increased. In diaphragms in which they do not occur spontaneously, they may be brought on by the addition of potassium to the bath to give a concentration of 0·085% KCl. When they are thus produced, they may be greatly enhanced by the addition of adrenaline. Once initiated in this way, the rhythmical activity continues for a considerable time even after washing with fresh solution. The rhythm has not been found in muscle after curare or denervation. It is therefore suggested that the rhythmical activity occurs at the region of the nerve endings.

Hypothalamic control of the secretion of adrenocorticotrophic hormone. By J. de Groot and G. W. Harris. *Physiological Laboratory, Cambridge*

Electrical stimulation of the posterior region of the tuber cinereum or of the mammillary body of unanaesthetized, unrestrained rabbits results in a lymphopenia which is similar to that following emotional stress. Stimulation of

other regions in the hypothalamus, or of the pituitary gland, does not elicit the response. The lymphopenic response which follows an emotional stress stimulus is abolished by lesions in the zona tuberalis (two cases), and in most cases abolished or diminished by transverse lesions in the posterior part of the tuber cinereum or mammillary body. Similar lesions in the posterior part of the pars distalis or pars intermedia, and lesions which interrupt the infundibular stem do not interfere with normal responses. It is concluded that secretion of the adrenocorticotrophic hormone (which is thought to be responsible for the lymphopenia following emotional stress) is under hypothalamic control via the hypophysial portal vessels of the pituitary stalk.

The use of suramin for the determination of the isoelectric points of some enzymes. By E. D. Wills and A. Wormall. *Department of Biochemistry, The Medical College of St Bartholomew's Hospital*

The trypanocidal drug suramin ('Antrypol' : Bayer 205) strongly inhibits many enzymes (Town, Wills & Wormall, 1949). Where an enzyme is inhibited by the drug in acid solution but not at pH's alkaline to the isoelectric point of the enzyme, the inhibition-pH curve usually falls sharply at about the isoelectric point (Wills & Wormall, 1949).

Further investigation has shown that inhibition tests with suramin often enable the isoelectric point of an enzyme to be determined. The method is simple and it does not require the purification of the enzyme. Few values for the isoelectric points of enzymes, as determined by electrophoresis, are given in the literature, but there is good agreement between our values and those which are recorded. Evidence of species differences have been obtained for carbonic anhydrase (values of pH 5·2–5·4 and 6·0–6·2 for the ox and sheep erythrocyte enzymes respectively) and for the catalases.

REFERENCES

Town, B. W., Wills, E. D. & Wormall, A. (1949). *Nature, Lond.*, **163**, 735.
Wills, E. D. & Wormall, A. (1949). *Biochem. J.* **44**, xxxix.

Some antigen-antibody reactions studied with the aid of radioactive isotopes. By T. E. Banks, G. E. Francis, W. Mulligan and A. Wormall. *Departments of Biochemistry and Physics, The Medical College of St Bartholomew's Hospital*

The precipitin reaction between various antigens and their antibodies has been studied quantitatively with the aid of antigens labelled with ^{32}P, ^{35}S or ^{131}I, and antibodies labelled with ^{131}I or ^{35}S. The use of these tagged reactants renders possible the determination of antigen and antibody in very small amounts of precipitate.

Evidence for the presence of antibodies capable of reacting with phospholipids has been obtained in experiments with the lipovitellin-antilipovitellin system in which the antigen was labelled with ^{32}P and the antibody with ^{131}I.

A study of the inhibition of the iodoprotein precipitin reaction by 3:5-diiodotyrosine labelled with ^{131}I has produced evidence in support of the view that the antibodies we have studied are univalent in the sense that each molecule has only one specific combining group for the corresponding antigen.

Forced expiration against an absolute resistance. By J. N. Mills.
Department of Physiology, University of Cambridge

If maximal expiration is made against an absolute resistance, abdominal pressure, recorded from a gastric balloon, rises with the mouth pressure.

If the lungs contain 2 l. or more above the residual air the mouth pressure equals and often exceeds the abdominal pressure. If the lungs are fairly empty, the abdominal considerably exceeds the mouth pressure.

Thoracic musculature can apparently only be used in forcible expiration if the lungs are full.

If the mouth pressure is held at about 40 mm. Hg, the abdominal pressure often falls to 10 or 20 mm. Hg, even if the cheeks are supported. Pressure recorded from a needle in the trachea also falls while the mouth pressure is maintained.

The reactions of the colonic circulation in man to adrenaline and noradrenaline By J. Grayson, *Department of Physiology, The University of Bristol*; and H. J. C. Swan, *Sherrington School of Physiology, St Thomas's Hospital*

The temperature of the bowel exposed on the surface of the abdomen following the operation of colostomy was recorded thermoelectrically, and used to indicate blood-flow changes (Grayson, 1949). In a series of nine experiments adrenaline was given intravenously, using a mechanically driven infusion apparatus. Doses of 5–10 μg./min. administered for periods of 3–5 min. caused a pronounced fall in temperature (1·0–1·5° C.) in every case, with sometimes a distinct pallor of the colostomy. Following the infusion in some cases the bowel temperature rose beyond its starting level before finally subsiding to base-line. Seven experiments were also performed with noradrenaline, which, in similar doses, produced similar results. Local administration of both these drugs also gave unmistakable evidence of vasoconstriction.

REFERENCE

Grayson, J. (1949). *J. Physiol.* **109**, 439.

The effect of limbering-up on the oxygen usage during track running. By H. R. NOLTIE. *Department of Physiology, School of Medicine, Leeds*

The expired air of three men, non-fasting, was sampled at intervals whilst running with nose clipped, at 2 min./lap, twelve times around a 440 yd. track. An assistant proffered a mouthpiece and collected a known number of breaths in Douglas bags over a fixed stretch. Each experiment was duplicated and weather conditions noted.

The oxygen usage, less resting, in the first lap of the standard run was significantly increased when the runner had just previously limbered-up by running 2 laps at 2½ min./lap; but not if 1½ min. had elapsed since limbering-up.

The oxygen usage of the subsequent laps fluctuated about a mean but was not significantly affected by the limbering-up; nor was the final oxygen debt. Limbering-up appears likely to have shortened the period of anaerobic work normally occurring at the start of this exercise, but further investigation is necessary.

Vascular responses of the breast to thermal stimulation. By V. R. PICKLES. *Physiology Department, Newcastle-upon-Tyne*

The instrument used is that of which a full account has been published.

Twelve normal subjects were used during the first fortnight after childbirth. The instrument and the stimuli were for practical reasons applied contralaterally, the latter (0–5 and 45–50° C., 5 cm. diam.) either to the areola or a control region below the clavicle, for periods of 1 min.

Hot stimuli to the areola caused vaso-dilatation, cold stimuli vaso-constriction; stimuli to the control region, often subjectively more intense, had no significant effect. For the whole group, the blood flow during and for 10 sec. after the hot stimulus averaged 7% ± 2·8 (S.E. of diff. of means) above that in the 25 sec. periods before and after; the cold stimulus caused a decrease of 7% ± 1·8. One subject consistently gave changes to 120 and 80% of the resting value.

REFERENCE

Pickles, V. R. (1949). *Quart. J. exp. Physiol.* **35**, 219.

Dextran as plasma substitute and blood-pressure homeostasis. By G. VAN DEN HEUVEL. *Institute of Pharmacology, University of Ghent*

We examined in experiments on dogs the effects of dextran infusions in circulatory collapse and shock induced by bleeding, on the arterial pressure and on the mechanisms of blood-pressure homeostasis. In dogs anaesthetized with

chloralosane, respiration and blood pressure from the femoral artery were registered. In some animals the cardio-aortic vagus nerves were cut in order to limit reflex blood-pressure regulation to the carotid sinus. Occlusion of both carotid arteries (low pressure in carotid sinus) induces reflex rise of general arterial pressure and liberates the total 'blood-pressure reserve'. By de-occlusion of carotid arteries arterial pressure returns to normal level. Bleeding of different amounts was done up to suppression of carotid sinus reflexes and exhaustion of the 'blood-pressure reserve'. After different durations of circulatory collapse, up to 1·30 hr. after bleeding, infusions of dextran solution were given. Control of blood-pressure and carotid sinus reflexes showed that not only arterial pressure returned to normal or close to normal levels, but also that the reflex blood-pressure homeostasis and the 'blood-pressure reserve' were restored.

Thermal responses of extremities to cold in relation to body temperature. By W. G. WILSON-DICKSON and C. H. WYNDHAM. *R.N. Physiological Laboratory, Alverstoke, Hants, and Climatic Research Unit, Department of Human Anatomy, Oxford*

Reflex vaso-constriction in hands and feet accounts partly for their disproportionately rapid cooling during exposure of the naked human body to cold (Freeman & Nickerson, 1938). Clothed subjects, when cooling, show similar effects. With body temperature normal, a cooled hand may show vaso-dilatation after immersion of the other extremities in hot water (Uprus, Gaylor, Williams & Carmichael, 1935; Uprus, Gaylor & Carmichael, 1936; Benatt & Taylor, 1940).

Our observations concern the possibility, under practical conditions down to −10° C., of preventing excessive cooling of extremities, especially hands. Maintenance of body temperature was found to override variations in gloves. Marked cooling of hands and feet, during body cooling at rest, was promptly reversed when body temperature was raised by light work, suggesting predominance of reflex vascular responses to body temperature.

This was confirmed in 10 min. exposures concurrent with severe work, in which bare hands remained warm. However, with the body scantily clad, some finger cooling occurred, perhaps contributed to by precooling of arterial inflow by venae concomites (Bazett, Love, Newton, Eisenberg, Day & Forster, 1948).

REFERENCES

Bazett, H. C., Love, L., Newton, M., Eisenberg, L., Day, R. & Forster, R. (1948). *J. appl. Physiol.* **1**, 3.
Benatt, A. & Taylor, H. J. (1940). *Brit. Heart J.* **2**, 21.
Freeman, H. & Nickerson, R. F. (1938). *J. Nutrit.* **15**, 597.
Newburgh, L. H. & Harris, M. (eds.) (1945). '*Clothing Test Methods*', National Research Council, U.S.A.
Uprus, V., Gaylor, G. B. & Carmichael, E. A. (1936). *J. clin. Sci.* **2**, 301.
Uprus, V., Gaylor, G. B., Williams, D. J. & Carmichael, E. A. (1935). *Brain*, **58**, 448.

Low threshold rods and the perception of blue. By E. N. Willmer.
The Physiological Laboratory, University of Cambridge

One-half of a circular field, subtending about 4° at the eye, is illuminated by light of any wave-band between violet and yellow (Ilford Spectral Filters) of sufficiently low intensity that the colour is indistinguishable to a thoroughly dark-adapted eye. This half therefore stimulates only the rods.

The other half is then illuminated by any wave-band between yellow and extreme red so that it appears coloured, and cones are presumably being stimulated. The 'rod' field immediately darkens and, after a momentary pause, appears blue, so that, by binocular matching, it produces the same hue sensation as a spectral band 465–470 mμ. When the 'cone' field is extinguished the 'rod' field brightens and its colour fades.

The blue colour cannot be complementary since it is independent of the wave-length of the 'cone' field. It must result from interacting rod and cone pathways.

Pulmonary vasomotor reactions in isolated perfused cat lungs in response to the inhalation of various mixtures of oxygen and nitrogen. By Helen Duke*. *Department of Physiology, Edinburgh University*

Isolated cat lungs under positive- or negative-pressure ventilation were perfused through the pulmonary artery with the animal's own heparinized blood at constant volume inflow.

Ventilation with pure O_2 (replacing atmospheric air) for periods up to 10 min. produced no pulmonary vascular response.

Vasoconstriction, shown by an increase in pulmonary arterial pressure, occurred after 1–2 min. ventilation with N_2 (replacing air) or 5% CO_2 in N_2 (replacing 5% CO_2 in air). This effect was independent of the mode of ventilation or of changes in tidal air. It was unaffected by the administration of ergotoxine or atropine or the choice of anaesthesia (nembutal or chloralose) for the preliminary dissection.

Ventilation with neon or H_2 also caused vasoconstriction.

These results support those of von Euler & Liljestrand (1946), Nisell (1948), and Motley, Cournand, Werko, Himmelstein & Dresdale (1947).

* Now at the Royal Free Hospital School of Medicine.

REFERENCES

Euler, U. S. von and Liljestrand, G. (1946). *Acta Physiol. Scand.* **12**, 279.
Nisell, O. (1948). *Acta Physiol. Scand.* **16**, 121.
Motley, A. L., Cournand, A., Werko, L., Himmelstein, A. & Dresdale, D. (1947). *Amer. J. Physiol.* **150**, 315.

Proprioceptors at the joint of the epiglottis. By B. L. Andrew.
Department of Physiology, University College, Dundee

Afferent nerve impulses can be detected in the superior laryngeal nerve of the rat under urethane anaesthesia and some of this impulse traffic arises from proprioceptors within the larynx (Andrew, 1949). The volume of afferent activity rises and falls with respiration as has been described by Petitpierre (1943) in the dyspnoeic rabbit. The greatest activity occurs during inspiration. The rhythmic element is contributed by the most cranial branch of the superior laryngeal nerve and can be tracked to the region of the thyro-epiglottic joint. The sensory endings are deployed at or near the joint and are connected to large myelinated nerve fibres. The endings are slowly adapting and are stimulated by tension produced in the joint either by the contraction during inspiration of muscles attached to the epiglottis or by the insertion of a needle point between the articulating surfaces.

Descent of the epiglottis produces a continuous discharge in several fibres.

REFERENCES

Andrew, B. L. (1949). *J. Physiol.* **110**, 21 *P*.
Petitpierre, C. (1943). *Helv. physiol. Acta*, **1**, 325.

The effect of adrenaline and noradrenaline on the rate of the denervated heart. By I. R. Innes and H. W. Kosterlitz. *Department of Physiology, University of Aberdeen*

In cats, under nembutal anaesthesia (45 mg./kg., intraperitoneally), both stellate and both middle cervical ganglia were removed and the vagi cut. In some animals, bilateral adrenalectomy was carried out acutely; in others, the right adrenal was removed and the left denervated, either acutely or 10–14 days previously.

The increase in the frequency of the heart beat was usually, but not always, smaller after injection into the femoral vein of L-noradrenaline than after equal doses of L-adrenaline (0·2–2 μg.). In confirmation of Rosenblueth and Schlossberg (1931) it was found that intramuscular injection of cocaine hydrochloride (8 mg./kg.) did not increase the response to adrenaline. On the other hand, the response to noradrenaline was markedly increased, in some experiments 3-fold. This phenomenon was not abolished by dibenamine.

REFERENCE

Rosenblueth, A. & Schlossberg, T. (1931). *Amer. J. Physiol.* **97**, 365.

The release of histamine by opium alkaloids. By P. A. NASMYTH and H. C. STEWART. *Physiology Department, St Mary's Hospital Medical School*

Morphine and codeine elicit the triple response (Sollmann & Pilcher, 1917; Lewis, 1927). A similar phenomenon can be produced in human skin with diamorphine, papaverine and pethidine when injected intradermally. No response was obtained in the skin of the guinea-pig, rabbit or rat with these drugs. In man the responses are reduced by antihistamine drugs.

In cats, intravenous morphine produces a sudden fall in blood-pressure which takes at least thirty minutes to return to normal (Schmidt & Livingston, 1933). This effect, preceded by a small rise in pressure, was confirmed in the normal and spinal cat.

Using the rat's diaphram preparation, histamine was shown to be liberated by D-tubocurarine (Rocha E. Silva & Schild, 1949). Exposure of this preparation to various opium alkaloids caused a similar release of histamine.

REFERENCES

Lewis, T. (1927). *Blood Vessels of Human Skin and their Responses.* London: Shaw and Sons, Ltd.
Silva, M. Rocha E. & Schild, H. O. (1949). *J. Physiol.* **109**, 448.
Schmidt, C. F. & Livingston, A. E. (1933). *J. Pharmacol.* **47**, 411.
Sollmann, T. & Pilcher, J. D. (1917). *J. Pharmacol.* **9**, 309.

Release of histamine by morphine alkaloids. By W. FELDBERG and W. D. M. PATON. *From the National Institute for Medical Research, Hampstead, London*

MacIntosh & Paton (1949) found that a number of organic bases, which liberate histamine in cats, cause the triple response in human skin. Morphine and codeine also elicit a triple response (Sollman & Pilcher, 1917; Lewis, 1927). Morphine alkaloids were, therefore, examined as possible histamine liberators in cats.

(1) Morphine and codeine, injected intravenously into cats, lower arterial blood pressure after a latency of 15–20 sec., a characteristic of histamine liberators; simultaneously, histamine appears in plasma but its concentration here remains lower than after propamidine for the same depressor effect. Histamine release, therefore, may not wholly account for the observed fall in blood pressure after morphine and codeine.

(2) When injected arterially into the isolated perfused gastrocnemius muscle of the cat, morphine, codeine, thebaine and apomorphine cause the appearance of several μg. histamine in the effluent. Papaverine has the same effect.

REFERENCES

Lewis, T. (1927). *Blood Vessels of Human Skin and their Responses.* London: Shaw and Sons, Ltd.
MacIntosh, F. C. & Paton, W. D. M. (1949). *J. Physiol.* **109**, 190.
Sollmann, T. & Pilcher, J. D. (1917). *J. Pharmacol.* **9**, 309.

Partition chromatography of adrenaline and noradrenaline. By ALEX COMFORT and JOHN L. D'SILVA. *Department of Physiology, London Hospital Medical College, E.* 1

James (1948) has described the separation of adrenaline and noradrenaline upon paper, using phenol as the running phase. The chromatogram was developed with 0·44% potassium ferricyanide in phosphate buffer at pH 7·8, which oxidizes the substances to the corresponding adrenochromes.

We have found that the sensitivity of the method can be increased by drying the developed chromatograms over an electric heater at an air temperature of about 100° C., and examining under the ultraviolet lamp, using a Wood's glass filter. The adrenochrome is decolorized, and converted to a yellow fluorescent substance, which is more readily detectable at low concentrations than the visible pigment. By this means, spots containing 2μg. of adrenaline or noradrenaline per cm.^{2} can be located as greenish fluorescent areas, which are stable on keeping.

REFERENCE

James, W. O. (1948). *Nature, Lond.*, **161**, 851.

Renal circulation and urine flow in the rabbit during severe asphyxia. By L. E. MCGEE and E. ULLMANN

The coronary circulation under stress. By J. P. SHILLINGFORD

PROCEEDINGS

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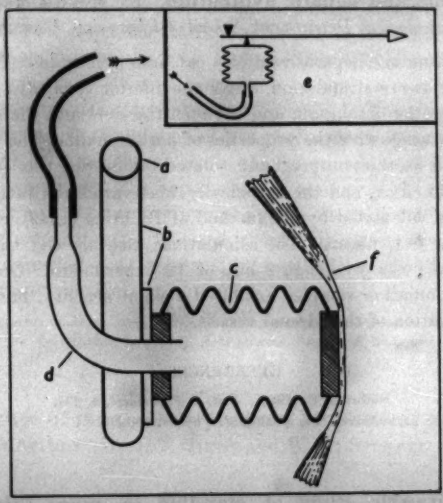
7 *January* 1950

A method of recording movements of the diaphragm. By C. A. DE CANDOLE, W. W. DOUGLAS and K. E. V. SPENCER. *A Ministry of Supply Establishment*

An independent record of movements of chest and diaphragm is often needed in studies of respiratory function; the investigation of paralysing agents which have a selective action on one or the other muscle group is a case in point.



The phrenograph in use (schematic). The instrument is shown as it lies against the diaphragm *f*. *a*, transverse brass support; *b*, vertical brass support and mounting plate; *c*, rubber bellows; *d*, spigot and transmission tube; *e* (inset), metal bellows pressure recorder.

An instrument, which has been named a phrenograph, has been devised which gives satisfactory records of the movements of the diaphragm in cats and rabbits. It consists of a small rubber bellows mounted on a rigid brass support (Fig. 1). With the abdomen opened in the mid-line and the hepatic ligament

cut, the phrenograph is introduced in such a way that the free end of the bellows lies in contact with the central tendon of the diaphragm.

The brass support to which the other end of the bellows is fixed is then securely clamped to the table. The variations in pressure which movements of the diaphragm set up are transmitted to a suitable instrument and recorded on smoked paper. The metal bellows recorder designed by Wilson (1949) has been used for this, as it is leak-proof and has an almost linear response over the required range.

The phrenograph is easily calibrated by clamping the bellows in a vertical position and placing weights on the free end.

REFERENCE

Wilson, K. M. (1949). Personal Communication.

Noradrenaline and hepatic sympathin. By Monica Mann and G. B. West. *Pharmacological Department, School of Pharmacy, University of London*

Blood taken from the hepatic vein of a cat under chloralose (D'Silva, 1936), during hepatic nerve stimulation, whilst the inferior vena cava is temporarily occluded above the diaphragm and between the liver and the adrenal veins, contains a substance with the properties of noradrenaline. The noradrenaline activity, tested on the non-pregnant isolated rat uterus, the isolated rectum of the week-old chick, and the chronically denervated nictitating membrane of the cat, and calculated by the method of Bülbring (1949), corresponds to 0·05 to 1·05 μg./ml. plasma. The calculations also showed that adrenaline ($<$0·003 μg./ml.) was present in 2 out of 12 experiments. Occasionally the control pre-stimulation samples exhibited a slight activity, but this was not reduced by ligation of the adrenal vessels.

REFERENCES

Bülbring, E. (1949). *Brit. J. Pharmacol.* **4**, 234.
D'Silva, J. L. (1936). *J. Physiol.* **85**, 219.

Electromyographic study of standing in man: thigh and leg muscles. By W. F. Floyd and P. H. S. Silver. *Department of Physiology and Department of Anatomy, Middlesex Hospital Medical School, London, W.* 1

Surface and concentric needle electrodes were placed over and into the muscles of the thigh and leg, and action potentials were recorded simultaneously from flexor and extensor muscles with the Ediswan Standard six-channel electroencephalograph. In the standing position, the activity recorded was found to

fluctuate continually. In a certain position of comfortable stance, electrical activity was practically zero in the muscles studied; we shall call this position the nul position. Small deviations from this position were constantly occurring during standing and were associated with bursts of flexor and extensor muscle activity, of such a pattern as to correct the deviation and return the body to the null position. This continual 'deviation correction' activity was exaggerated when the eyes were closed.

The significance of neuromuscular paralysis in acute poisoning of rabbits with tetraethylpyrophosphate (TEPP). By C. A. DE CANDOLE, W. W. DOUGLAS and K. E. V. SPENCER. *A Ministry of Supply Establishment*

After intravenous injection of tetraethylpyrophosphate into rabbits (0·3 mg./kg.) the volume of air inspired drops precipitately despite initially augmented costal breathing. The muscular power, first of the diaphragm and then of the chest, is diminished, and the respiratory intrapleural pressure differences are reduced. Circulatory changes are comparatively slight initially. The fact that diaphragm and chest are unequally affected suggests a peripheral component in the mechanism whereby muscular activity is impaired. The response of the diaphragm to repetitive stimulation of the phrenic nerve becomes small and unsustained, while direct stimulation of the muscle still elicits tetanic responses of normal type. The impairment of activity is therefore probably due to an action of the tetraethylpyrophosphate at the myoneural junction. Suppression of ventilation is followed shortly by respiratory and cardiac arrest, and it is concluded, as suggested by Lundholm (1949) that anoxia contributes significantly to this.

REFERENCE

Lundholm, L. (1949). *Acta physiol. Scand.* **16**, 345.

The influence of the osmotic pressure of the meal on gastric function. By J. N. HUNT and W. R. SPURRELL

The influence of the volume of the meal on gastric function. By J. N. HUNT and I. MACDONALD

Observations on the reliability of Albanese and Lein's method for estimation of sodium in urine. By A. A. G. LEWIS and G. L. S. PAWAN

Effect of posterior pituitary extracts on urinary excretion of sodium and chloride in man. By T. M. Chalmers, A. A. G. Lewis and G. L. S. Pawan

Oxygen carriage in subjects with venous-arterial shunts. By J. Ernsting and R. J. Shephard

Some properties of the isolated and unanaesthetized cat's cerebral cortex. By B. Delisle Burns

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY

1–2 *July* 1949

Cells of origin of proprioceptive fibres from eye-muscles. By S. Cooper, P. M. Daniel and D. Whitteridge. *Nuffield Department of Surgery, and the University Laboratory of Physiology, Oxford*

An attempt has been made to find the cells of origin of nerve fibres from the proprioceptive endings in extrinsic eye muscles.

Afferent discharges in the nerve to the inferior oblique muscle have been recorded (Cooper, Daniel & Whitteridge, 1949), and the present investigation is an extension of this work.

A needle electrode mounted on a Beattie-Souttar stereotaxic instrument was used to search the mid-brain of the goat for the cell bodies. The six extrinsic eye muscles were detached from the globe, and threads were attached to their freed extremities. The needle electrode was driven into the oculomotor nuclei and adjacent regions; the various eye muscles were then pulled.

When the needle tip was in the neighbourhood of the oculomotor nucleus records were obtained of afferent discharges with rates rising to 150 to 350 impulses per sec. as eye muscles were passively stretched. The technique of localizing the oculomotor nucleus will be demonstrated.

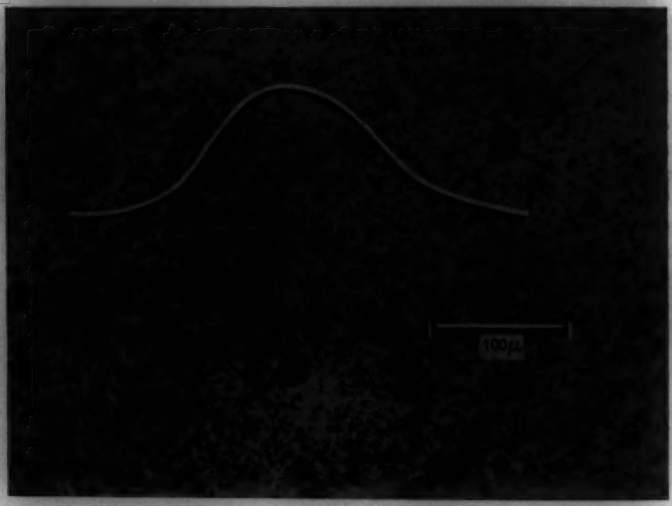
REFERENCE

Cooper, Sybil, Daniel, P. M. & Whitteridge, D. (1949). *J. Physiol.* **108**, 41*P*.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY
18 *February* 1950

Giant ganglion cells in the cat's retina. By W. A. H. Rushton.
Nobel Institute for Neurophysiology, Stockholm

In nearly all work with micro-electrodes upon the retina, the eye is opened, the lens removed, and the record taken from some suitable spot on the vitreal aspect of the retina. It is a matter of considerable importance to know what is the structure or set of structures whose activity is thus directly recorded.



Granit (1941), in earlier work on the frog, obtained evidence suggesting that optic nerve fibres were the structures involved, though the blind spot itself was rather silent. In the cat, however, conditions are different, for the spikes are larger, are maintained despite small eye movements, and are easily obtained well isolated from other large spikes (though records commonly contain some small spikes). These properties have contributed to make the large retinal spike

in the cat the preparation of choice for Granit and his colleagues during the past few years.

Now if the eviscerated eye is illuminated and observed through a low-power microscope, the retinal vessels may be seen very clearly against the green tapetum. In this way the movement of the micro-electrode may be observed relative to the retina. When the electrode is in position to record a large spike, it may be moved some 0·1 mm. in any direction without the spike falling to less than half value. An electrode recording from a nerve fibre would hardly be so indifferent to the direction of movement. The active structure is therefore more likely to be a large nerve cell or group of cells. Since puncture with a fine needle often causes the large spike to vanish all → none, and never to diminish step-wise, the 'element' in the cat appears to be a large single cell. The question now arises: 'Are there large cells in the ganglion layer, and are they about as common as the places whence large spikes may be found?'

This demonstration shows flat preparations of the whole retina of the cat (and rabbit) stained by a modification of Palmgren's silver-pyrogallol method (1948). There is seen to be a sprinkling of large cells with many branching processes. In some cases one of these processes (unbranched) is seen to be an optic nerve fibre. The cell with its branches embraces an area of some 1 mm.^{2} If cell and processes discharged more or less synchronously it might account for the observed spatial spread. The figure shows the ganglion cell layer in the cat, the horizontal line representing 100μ. The distance of the inked curve above this horizontal shows how the recorded spike height varied as the electrode moved straight across the retina. The curve though taken from a different eye, indicates roughly the extent of spatial distribution.

I have never found more large-spike elements than large cells in any given part of the retina (when properly stained). Occasionally the correspondence seemed rather exact, but usually the cells were more numerous than distinct responding 'elements'. But since many 'elements' will not respond in the conditions of intense illumination employed, such a discrepancy is to be expected.

Upon these grounds, and those recently summarized elsewhere (Rushton, 1949), it seems likely that the large ganglion cells are responsible for the large retinal spikes in the cat.

REFERENCES

Granit, R. (1941). *Acta physiol. scand.* **1**, 370.
Palmgren, A. (1948). *Acta Zool.* **29**, 378.
Rushton, W. A. H. (1949). *Nature, Lond.*, **164**, 743.

A reaction time meter for students' use. By L. BERNSTEIN. *Physiology Department, London Hospital Medical College*

This instrument was designed for use by medical students for experiments on the physiology of reaction and learning.

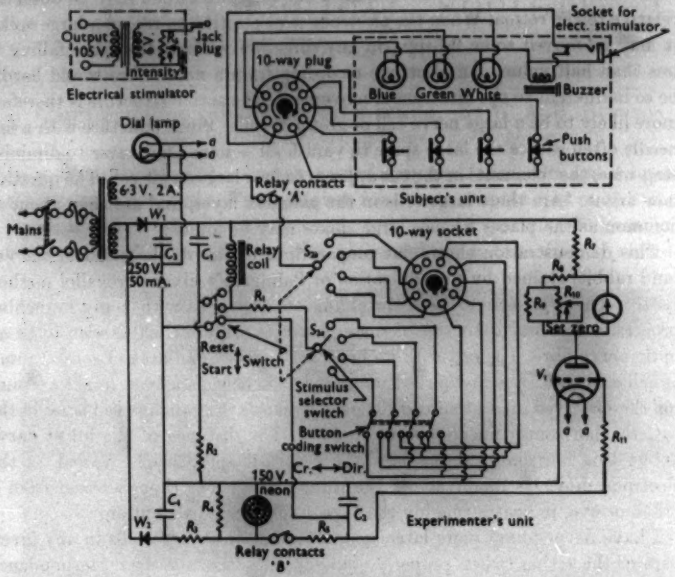


Fig. 1. Circuit diagram.

W_1, W_2, selenium oxide rectifiers 250 V., 30 mA.; R_1, 330 kΩ., ½ W.; R_2, 1·5 MΩ., ½ W.; R_3, 50 kΩ., ½ W.; R_4, 100 kΩ., ½ W.; R_5, 16·4 MΩ.; R_6, 2 kΩ., wire-wound var.; R_7, 25 kΩ., 2 W.; R_8, R_9, 8 Ω., ½ W.; R_{10}, 30 Ω., wire-wound var.; R_{11}, 1 MΩ., ½ W.; C_1, 0·05 μF., 350 V.; C_2, 1·0 μF., 350 V.; C_3, 8 μF., 350 V. elect.; C_4, 0·05 μF., 350 V.; V_1, 6J5G. Relay—G.P.O., coil 20,000 Ω., contacts 2 make.

It consists of two units, linked by a multiway plug and cable. The experimenter's unit is a device for selecting and presenting a stimulus to the subject, and for measuring the time which passes before he makes the appropriate response. The subject's unit is a small box on which are mounted three coloured lamps and buzzer—these provide the stimuli, visual and auditory—and a set of four push-button switches. The subject must press the correct button to extinguish the stimulus and stop the time-measuring device. Provision is made in the experimenter's unit for the stimuli and push-buttons to be interconnected in two different ways, one so that the relation is obvious ('Direct switching')

the other less apparent ('Crossed switching'). Thus a simple, or a more difficult, problem can be set.

Operation of the circuit (refer to circuit diagram). The power supply is from the 230 V. a.c. mains, and provides two h.t. supplies at +300 and −300 V. to ground.

Valve V_1 has zero bias and passes enough current to cause full-scale deflexion of the meter. The meter is mounted inverted, so that the needle rests at the left-hand end of the scale. The scale is calibrated directly in seconds and tenths of seconds.

A current of 1·0 mA. flows through R_1, switch S_{2a}, and the relay coil, the circuit being completed by one of the normally closed push-buttons in the subject's unit, the button being chosen by the setting of S_{2a}. This standing current is not enough to close the relay. When the switch S_1 is moved to the 'Start' position the charge on C_1 is sent through the relay and closes it. The standing current through R_1, etc., then holds it closed.

The closing of the relay completes two circuits together:

(*a*) the '*B*' contacts apply a −150 V. d.c. neon-stabilized voltage, derived from the −300 V. h.t., to the condenser C_2, through a charging resistor R_2.

(*b*) the '*A*' contacts apply 6·3 V. a.c. through the switch S_{2b} to the chosen stimulator (lamps or buzzer).

As C_2 charges it biases V_1 grid. The plate current falls, and the meter needle moves from left to right over the scale. When the subject presses the correct push-button on his unit, the relay holding circuit is broken, the relay opens, and the circuits through the '*A*' and '*B*' contacts are both broken. The stimulus is extinguished, and the meter needle comes to rest. The meter reading indicates charging time for C_2, and this is the Reaction Time.

When S_1 is moved back to the 'Reset' position the bias on V_1 grid is short-circuited and the meter needle returns to the zero setting.

A small electrical stimulator has been constructed to provide a skin stimulus. This plugs into a socket provided on the subject's unit. No explanation of this, other than that given in the circuit diagram, is necessary.

An intravenous infusion apparatus with automatic warning devices.

By H. T. Howat and B. Schofield. *Department of Physiology, University of Manchester, and the Buckston Browne Research Farm*

The apparatus has been designed for use in the double histamine test for inhibitors of gastric secretion (Howat & Schofield, 1948). It delivers a volume of 20 ml. at a constant rate over 45 min., and the test includes two such injections of a histamine solution.

The plunger of a 20 ml. all-glass syringe is driven by a screw which is turned by a rotating cage. A synchronous motor revolves the cage at constant speed.

This method of driving a syringe was described by Last (1945). Interruption of the infusion unless immediately righted impairs the value of the test, and the usefulness of the apparatus is increased by fitting alarms which give immediate warning of failure.

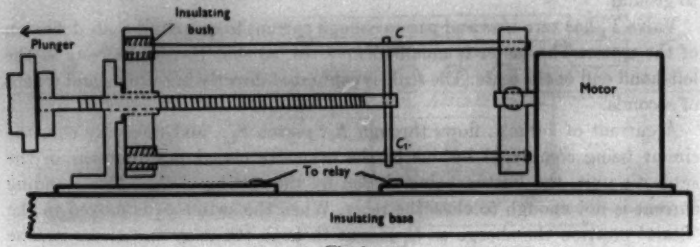


Fig. 1.

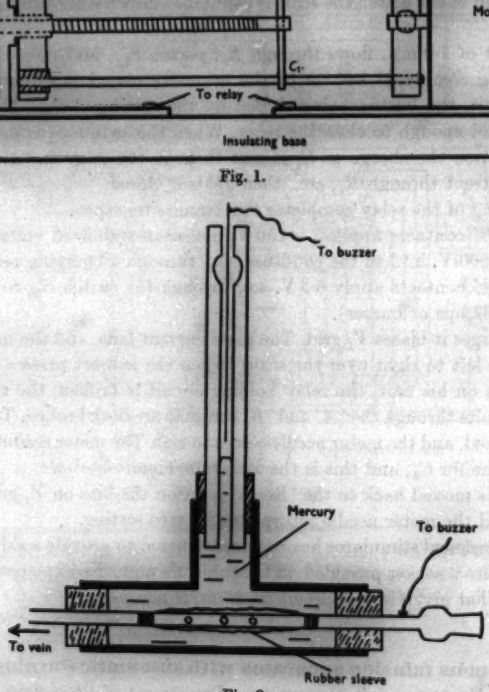


Fig. 2.

Failure may occur in two ways.

(1) The plunger may stick. This is infrequent with a lubricated syringe of good quality.

(2) The vein may kink, or clotting may occur. The rise in injection pressure usually does not stop the infusion at once, but may lead to a leak past the plunger. In view of the slow rate of injection this can cause serious loss. Warning devices operate in both situations.

(1) *Sticking plunger.* If the force required to turn the screw becomes too great, the motor reverses. The cage is insulated from the screw except at the points of contact with the cross-bar, C, C_1 (Fig. 1). When these contacts are broken on reversal the current through a relay is interrupted and a buzzer sounds.

(2) *Raised injection pressure.* A pressure gauge is used (Fig. 2). The solution passes from the syringe through a stainless steel needle of large bore. A thin latex rubber sleeve is slipped over the needle and tied at either end so that it covers the middle two-thirds. The space so enclosed by the tubing communicates with the lumen of the needle through three small holes. The needle is fixed by small rubber plugs in the horizontal limb of an inverted T-piece, which is filled with mercury. Finally, a piece of capillary bore tubing is inserted into the vertical limb of the T-piece. A rise in pressure of the infusion distends the rubber sleeve and displaces the mercury level in the capillary. At a suitable level it makes contact with a platinum wire and completes the circuit to the buzzer.

The advantages of this unit are:

(1) It is simple and compact.

(2) It is operated by a minimal volume change and therefore responds quickly, in spite of the slow rate of flow.

(3) The injected solution is only in contact with rubber and stainless steel.

REFERENCES

Howat, H. T. & Schofield, B. (1948). *J. Physiol.* **107**, 30*P*.
Last, C. E. (1945). *Brit. med. J.* **1**, 122.

Metal bellows recorders.

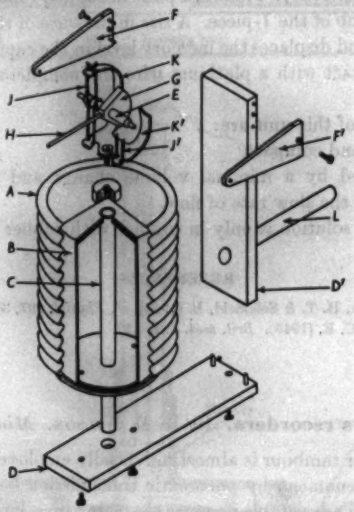
By K. M. Wilson. *Ministry of Supply*

The ordinary rubber tambour is almost universally employed for the recording of physiological phenomena by pneumatic transmission but possesses several inherent faults, such as unstable pressure characteristics, large volume changes, tendency to develop leaks and poor response to rapid pressure variations.

During recent years, flexible metal bellows have found widespread application in industry and are now readily available from commercial sources. In the author's laboratory, recording instruments incorporating such bellows have been in continuous use during the past three years and have proved much superior to rubber tambours for the majority of purposes, since they are mechanically robust, maintain their calibration permanently and produce a more faithful record of pressure variations in the system under examination.

A satisfactory design of high sensitivity and simple construction is shown in the accompanying 'exploded' drawing. In order to reduce 'dead space',

the bellows (*A*) is largely filled by the closed hollow cylinder (*B*) through which the inlet tube (*C*) passes, all components being soldered together to form an airtight system which is attached to the frame (*D*, *D'*) by two screws. The spindle (*E*) is provided with clock-type pivots working in the side plates (*F*, *F'*) and carries a balance weight (*G*) and wire spigot (*H*) to which a straw lever (not shown) can be attached. The spindle (*E*) is caused to rotate with movements of the bellows by the silk loops (*J*, *J'*) which are wrapped once round the spindle and held taut by the C springs (*K*, *K'*). A short $\frac{3}{8}$ in. diam. rod (*L*) is screwed in the frame to facilitate attachment to the kymograph, and alternative tapped holes can be provided to allow of the instrument being used in the horizontal position if required.



Detailed dimensions are not given since they are dependent on the type of bellows used and the sensitivity required. In the recorder demonstrated, the uncompressed length of the bellows and the diameter are both $2\frac{3}{8}$ in., while the spindle is $\frac{1}{8}$ in. diameter. When used with a 24 cm. lever, the full-scale deflexion is 15 cm., corresponding to a pressure differential of 15 cm. water.

The system is completely free from back-lash and possesses good internal damping, but additional air damping can be obtained by gluing a suitable feather along the length of the straw lever.

An oscilloscope for the examination of slow recurrent transient waveforms and bio-electric potentials. By L. BERNSTEIN. *Physiology Department, London Hospital Medical College*

Commercial oscilloscopes can be used for the display of the more rapid bio-electric phenomena, such as action potentials, but for e.c.g., e.e.g., or phonocardiagram display a slower time base than these oscilloscopes provide is desirable. A simple oscilloscope is described which can be used for the display of such potentials, either for demonstration purposes, or for monitoring during the making of permanent records.

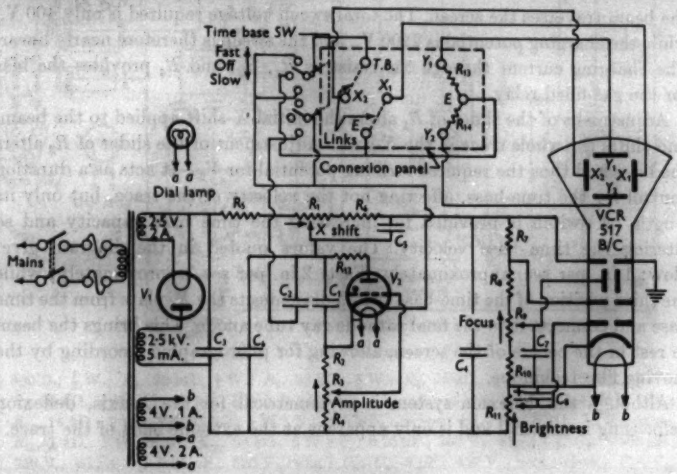


Fig. 1. Circuit diagram.

R_1, 2 00 kΩ., carbon. var.; R_2, 56 kΩ., ½ W.; R_3, 100 kΩ., carbon. var.; R_4, 10 MΩ., 1 W.; R_5, 680 kΩ., ½ W.; R_6, 100 kΩ., ½ W.; R_7, 680 kΩ., ½ W.; R_8, 560 kΩ., ½ W.; R_9, 500 kΩ., carbon. var.; R_{10}, 150 kΩ., ½ W.; R_{11}, 50 kΩ., carbon. var.; R_{12}, 1 kΩ., ½ W.; R_{13}, R_{14}, 3·9 MΩ., $\frac{1}{16}$ W.; C_1, C_2, 1 μF., 600 V., wkg.; C_3, C_4, 0·25 μF., 3·5 kV., wkg.; C_5, 4 μF., 600 V., wkg.; C_6, 2 μF., 2·5 kV., wkg.; C_7, 0·25 μF., 600 V., wkg.; C_8, 8 μF., 50 V., wkg.; V_1, 2X2; V_2, GT1C.

The circuit is shown in Fig. 1. The VCR517B cathode-ray tube is a tube having an instantaneous blue-green fluorescence, and a long persistent yellow phosphorescence of high intensity. The power supply is conventional except that the earthing point is 1000 V. −ve to h.t. +.

The time base is of the condenser charge type, using a gas-filled triode as the discharging relay. The time-base output voltage is taken from the cathode of this valve and is directly coupled to the operative deflector plate X_2. This

obviates the use of long-time-constant coupling circuits. The time base condenser C_1 charges from the high potential between the slider of the potentiometer R_1 and the h.t. −ve rail. This potential is variable between 2300 and 2100 V. by adjusting R_1. In the further discussion it will be assumed that the slider has been set so as to provide a P.D. of 2200 V. At the commencement of the charging cycle the condenser C_1 is uncharged, and its two plates are equipotential. The X_2 deflector plate is connected to the lower side of C_1 and is 200 V. positive to the final anode of the cathode-ray tube. This shifts the beam to the left-hand edge of the screen. As C_1 charges, X_2 deflector plate becomes less positive and then negative with respect to the final anode, and the beam traverses the screen. The total sweep voltage required is only 400 V., while the charging potential is 2200 V., and the sweep is therefore nearly linear. The charging current through the resistors R_2, R_3, and R_4 provides the bias for the gas-filled relay.

Adjustment of the slider of R_1 alters the initial X-shift applied to the beam, and shifts the whole trace in the X-axis. Adjustment of the slider of R_3 alters the bias, and thus the required striking potential for V_2. It acts as a duration control for the time-base, affecting not the velocity of the trace, but only its length. A switch is provided for increasing the time base capacity and so altering the time base velocity. The values quoted in the diagram give: Slow: 1 in. per sec. approximately; Fast: 2 in. per sec. approximately; while the third position of the time-base switch disconnects the X_2 plate from the time base and connects it to the final cathode-ray tube anode. This brings the beam to rest in the centre of the screen, allowing for photographic recording by the moving film technique.

Although the deflexion system is asymmetrical for the X-axis, deflexion defocusing is minimal and is only apparent at the extreme ends of the trace.

An electronic square wave stimulator.

By L. Bernstein.
Physiology Department, London Hospital Medical College

This instrument operates from the 230 V. a.c. mains. It provides a negative-going square wave-form output pulse, whose repetition rate, duration and current content may be separately varied. The range of values obtained is as follows:

Rate: repeated pulses from 1 in 5 sec. to 1000 per sec. in twelve steps—or single pulses.

Duration: from 100 μsec. to 50 msec. in nine steps.

Current: from 0 to 20 mA. peak.

In the model demonstrated the generator acts as a high-impedance source giving constant current into widely varying load resistances. This is advan-

tageous when it is difficult to ensure perfect electrode contact with a tissue to be stimulated. Its disadvantage is the deformation of the pulse which may be produced by the capacity of the load. A low-impedance generator does not have this disadvantage, but it supplies constant voltage and not constant current. A method is suggested for modifying the circuit, if desired, so as to provide either at will.

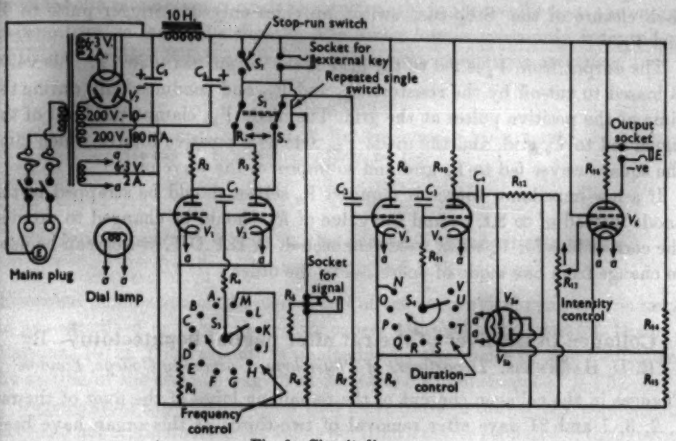


Fig. 1. Circuit diagram.

R_1, 800 Ω., ½ W.; R_2, 32 kΩ., ½ W.; R_3, 32 kΩ., ½ W.; R_4, 10 kΩ., ½ W.; R_5, 22 kΩ., ½ W.; R_6, 39 kΩ., ½ W.; R_7, 10 kΩ., ½ W.; R_8, 100 kΩ., 1 W.; R_9, 10 kΩ., 1 W.; R_{10}, 47 kΩ., 1 W.; R_{11}, 18 kΩ., ½ W.; R_{12}, 32 kΩ., ½ W.; R_{13}, 0·5 MΩ., carbon. var.; R_{14}, 6 kΩ., 12 W., w.w.; R_{15}, 1 kΩ., 2 W., w.w.; R_{16}, 68 kΩ., 2 W.; C_1, 0·25 μF., 350 V., wkg.; C_2, C_3, 0·002 μF., 350 V., wkg.; C_4, 0·25 μF., 350 V., wkg.; C_5, C_6, 8 μF., 450 V., wkg., elect.; V_1+V_2, V_3+V_4, 6SN7, G.T.; $V_{5a}+V_{5b}$, 6H6; V_6, 6V6, G.T.; V_7, 6X5, G.T. *A*–*B*, 9·5 MΩ.; *B*–*C*, 3·3 MΩ.; *C*–*D*, 1·7 MΩ.; *D*–*E*, 1 MΩ.; *E*–*F*, 330 kΩ.; *F*–*G*, 180 kΩ.; *G*–*H*, 120 kΩ.; *H*–*J*, 33 kΩ.; *J*–*K*, 15 kΩ.; *K*–*L*, 6·8 kΩ.; *L*–*M*, 1·5 kΩ.; *N*–*O*, 82 kΩ.; *O*–*P*, 330 kΩ.; *P*–*Q*, 220 kΩ.; *Q*–*R*, 330 kΩ.; *R*–*S*, 1 MΩ.; *S*–*T*, 2·2 MΩ.; *T*–*U*, 4·2 MΩ.; *U*–*V*, 12·6 MΩ.

Circuit operation (refer to Fig. 1). V_1 and V_2 act as a cathode-coupled multivibrator producing narrow, positive-going, square pulses at V_2 anode. The rate of oscillation is controlled by switch S_3, and the rates provided are: *A* 0·2, *B* 0·5, *C* 1, *D* 2, *E* 5, *F* 10, *G* 20, *H* 50, *J* 100, *K* 200, *L* 500, *M* 1000, per sec. The 'stop-run' switch, S_1, breaks or makes the h.t. to V_1 and V_2. A jack socket is paralleled with this switch so that an external key (i.e. drum contacts) can be used if desired.

The square pulses are differentiated into spikes by C_2 and R_7, and these are then fed to the grid of V_3. Each positive-going spike triggers V_3 and V_4, which form a cathode-coupled 'flip-flop'. A positive-going square pulse is produced

at V_4 anode for each spike arriving at V_3 grid. The duration of these square pulses is controlled by switch S_4, and the durations provided are: *N* 100 μsec., *O* 200 μsec., *P* 500 μsec., *Q* 1 msec., *R* 2 msec., *S* 5 msec., *T* 10 msec., *U* 20 msec., *V* 50 msec.

Switch S_2 selects repeated or single stimuli. In the 'Repeated' position V_1 and V_2 are in action; in the 'Single' position V_1 and V_2 are inoperative, and each closure of the 'Stop-run' switch provides only one trigger pulse to V_3 and V_4.

The output from V_4 is fed to the grid of the output pentode V_6. This valve is biased to cut-off by the resistors R_{14} and R_{15} and conducts only during the time of the positive pulses at the grid. The diode V_{5a} clamps the level of the signal fed to V_6 grid, and the diode V_{5b} acts as a positive limiter which clips the square waves fed to V_6 grid and so improves the waveform.

If a low-impedance source is required V_6 screen should be strapped to the anode instead of to h.t.+, and the value of R_{15} should be changed to provide the correct bias for V_6 when triode connected. A D.P.D.T. switch can be used to change from one mode of operation to the other.

Collagen in the liver of the rat after partial hepatectomy. By R. D. Harkness. *Department of Physiology, University College, London*

Changes in the collagen content of the remaining lobes of the liver of the rat 1, 2, 3, 7 and 21 days after removal of two-thirds of this organ have been investigated. Collagen was estimated by a modification of the method of Lowry, Gilligan & Katersky (1941), by estimation of nitrogen instead of by weight.

The percentage of collagen in the remaining lobes of the liver fell progressively in the first 2 days after partial hepatectomy; thereafter it rose slowly, but was still only about 70% of normal at 21 days.

The total quantity of collagen in the remaining lobes stayed constant for the first 2 days and later rose gradually to reach at 21 days about 65% of the quantity originally in the whole liver. The rise probably began on the third day.

REFERENCE

Lowry, O. H., Gilligan, D. R. & Katersky, E. M. (1941). *J. biol. Chem.* **139**, 795.

The nature of the weight loss following parturition and 'pseudo-parturition' in the mouse. By A. D. Dewar and W. H. Newton. *Department of Physiology, University of Edinburgh*

Following parturition there is a decrease in maternal body weight in addition to that due to the loss of foetuses and adnexa. A similar fall (10–15% body weight) occurs during the 2–3 days following pseudo-parturition (delivery of

placentae at term after earlier destruction of foetuses (Newton, 1935)). The nature of this loss has been investigated using the method of total metabolism (Dewar & Newton, 1948). The greater part of the weight loss (*c.* 80%) is due to water, brought about by a reduction in intake without an equivalent fall in excretion (relative polyuria). The remainder is accounted for by an imbalance between the foodstuffs eaten and those metabolized and excreted. Alterations in metabolic rate do not contribute significantly to the weight loss; the energy expenditure, in fact, falls slightly. The water loss is apparently independent of tissue loss since it can occur at the same time as nitrogen retention.

REFERENCES

Dewar, A. D. & Newton, W. H. (1948). *Brit. J. Nutrit.* **2**, 123.
Newton, W. H. (1935). *J. Physiol.* **84**, 196.

Bronchomotor fibres in the cervical sympathetic nerve.

By M. DE BURGH DALY and L. E. MOUNT

Electrical stimulation of the caudal end of the cut cervical sympathetic nerve in anaesthetized or decerebrate cats causes a bronchodilatation (cf. Dixon & Ransom, 1912). This response is unaffected by the choice of anaesthetic, and by section of the cervical vagi or of the rami communicantes (T1–T5).

Degeneration experiments show that these fibres in the cervical sympathetic nerve do not originate from the vagus nor from a source cephalad to the superior cervical ganglion, but issue from the thoracic sympathetic outflow. They relay in the stellate and middle cervical ganglia.

The majority of the postganglionic fibres run in the thoracic vagosympathetic trunk. No evidence has been obtained of postganglionic fibres in the cervical sympathetic nerve.

The bronchodilatation is unaffected by ergotoxine and by eserine, but is enhanced by ephedrine and cocaine, suggesting that the fibres are adrenergic in nature.

REFERENCE

Dixon, W. E. & Ransom, F. (1912). *J. Physiol.* **45**, 413.

Bronchial hypersensitization and hyposensitization in man.

By H. HERXHEIMER, *University College Hospital, London*

An attack of mild pollen asthma of the same intensity could not be induced repeatedly by the same amount of inhalant. If an increased amount was given, an attack of the previous intensity could be induced. By gradually increasing the amount of inhalant a state could be reached in which the subject no longer

responded to large amounts of pollen extract. This asthmatic subject therefore appeared to be hyposensitized. When the same was attempted with other subjects, the asthma became worse after the first attack in some, and they subsequently reacted to much smaller amounts of antigen and more violently. Only a small fraction of the initial amount would then be sufficient to induce a mild attack. This stage is believed to be equivalent to hypersensitization due to an excessive initial dose of antigen. If now the inhaled amount was increased gradually, hyposensitization could be achieved as in the first subject.

Overlap of cortical motor areas. By E. G. T. Liddell and C. G. Phillips. *University Laboratory of Physiology, Oxford*

The motor areas of three baboons (*Papio papio*) have been examined under hexobarbitone narcosis. Square current pulses (duration 5 msec.; strength 1–3 mA.; frequency 1 cyc./sec.) flowed between stigmatic cathode and indifferent anode, with a minimal interval of 1 min. between successive stimulations.

With weakest shocks the middle third of precentral gyrus only responds, yielding solitary movements of crossed thumb and index. With stronger shocks this thumb area is larger, and a contiguous area, centred on the medial third of the gyrus, yields crossed hallux twitches. With further strengthening, the crossed lower face twitches as well as the thumb when the lateral third is stimulated. With still stronger shocks, these three areas overlap to cover most of the motor area.

No other movements are obtained unless the pulse frequency is increased, when this simple pattern dissolves into the classical motor map.

Inhibition of experimental gastric secretion by histamine antagonists. By D. R. Wood. *Department of Pharmacology and Therapeutics, University of Sheffield*

The inability of antihistamine substances to inhibit the stimulant effect of histamine on gastric secretion is well known. They may even increase the secretory response to histamine. In the present experiments in anaesthetized cats, the gastric secretory response to continuous intravenous infusion of histamine has been reduced or abolished by phenergan, neoantergan or antistin, injected retrogradely into the ligated splenic artery so that a high concentration of the substance reaches the gastric mucosa. Injection of saline or of 3300 R.P., chemically similar to phenergan but without antihistamine activity, has no effect. If injected intravenously, in the doses used (5–25 mg.), antihistamine substances either have no action or cause an increased secretion rate. Although

there is a considerable fall of blood pressure on intravenous injection of these doses of phenergan, etc., there is no fall if they are injected into the splenic artery, as described above.

The sensitivity of the cold and warmth vaso-constrictor responses.

By J. Grayson. *From the Department of Physiology, University of Bristol*

Cutaneous vasoconstriction occurs reflexly on exposure to environments colder or warmer than the body (Grayson, 1949). In twenty-five human subjects cold stimulus was removed by covering completely with blankets. The skin temperature of the right index finger, which was left exposed, rose in every case to about 35° C. without rise in rectal temperature (both measured thermoelectrically). Subsequent exposure of the head and right arm usually produced less than 2° C. drop in finger temperature. Further vaso-constrictor responses were elicited by exposing the trunk or limbs to temperatures above or below 37° C. Raising the rectal temperature always reduced the sensitivity of these responses; a rise of 1° C. abolished them altogether. In three apparently normal subjects there was a marked hypersensitivity of the cold constrictor response. In these cases exposure of the head alone always caused the skin temperature to fall rapidly to 20 or 25° C.

REFERENCE

Grayson, J. (1949). *J. Physiol.* **109**, 53.

PROCEEDINGS
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A controlled temperature plethysmograph for the index finger. By A. D. M. GREENFIELD and J. T. SHEPHERD. *From the Department of Physiology, The Queen's University of Belfast*

This plethysmograph was designed for an investigation in which it was desired to make observations on the finger at controlled temperatures, and with various heads of external pressure up to 35 cm. of water. Essentially it consists

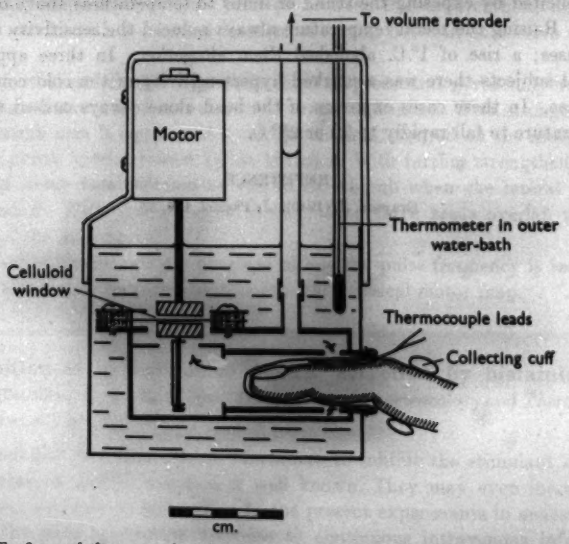


Fig. 1. The finger plethysmograph and surrounding water-bath. The driving magnet (upper) and driven magnet (lower) are shown in cross-hatching. The fingerstall is drawn separated from the finger, but in practice the external water pressure holds them in contact.

of a copper tube, 2·5 cm. external and 2·2 cm. internal diameter, and 6·5 cm. long (Fig. 1). A thin and loosely fitting fingerstall is attached to the circumference of the open end of the tube and is invaginated into it by the finger,

which is normally inserted up to the proximal interphalangeal joint. A thermo-electric junction is inserted between the fingerstall and the finger just proximal to the nail, to enable the temperatures at the surface of the finger to be measured. A 7 mm. diameter outlet communicates with a vertical glass tube. By adjusting the water-level in this tube varying external pressures can be applied to the finger.

The remainder of the apparatus is concerned with stirring the water between the copper tube and fingerstall, and providing a stirred water-bath to surround the plethysmograph and accessories. The copper tube is enclosed in a rectangular thin copper box, 9·5 by 3·75 by 3 cm., in the roof of which at one end is a celluloid window. Below the window is a small horizontal magnet rotating on a vertical axis. Baffles are arranged so that when rotating the magnet acts as a centrifugal pump, and circulates water along the copper box to enter the copper tube by a ring of holes near the open end. The motive force for this magnet is provided by a second similar and co-axial motor-driven magnet just above the window. The rotation of the top magnet also stirs the water in the surrounding water-bath. In this way the water inside and outside the plethysmograph is stirred, but there is no communication between the two systems.

The horizontal axis of the copper tube is 3·5 cm. above the bottom of the outer bath, and the external end protrudes slightly close to one corner. The tube is set at an angle of 15° to the adjacent side. This provides a comfortable angle of separation between the index and middle fingers, while permitting adequate water circulation all round the plethysmograph. Separate plethysmographs are required for the right and left sides. The walls of the outer bath are insulated with cork 4 mm. thick to protect the thumb and middle finger from its thermal effects, and to reduce heat exchange.

The collecting cuff is applied round the base of the finger.

The absorption of work when a muscle is stretched. By B. C. Abbott, X. M. Aubert and A. V. Hill. *Biophysics Research Unit, University College, London*

When a stimulated muscle is stretched during the contraction phase of a twitch a substantial part of the work done in stretching it disappears, i.e. is accounted for neither as heat nor as elastic potential energy. During the relaxation phase, however, the work done in a stretch may appear entirely as heat. When a muscle is stretched slowly by a constant load during a maintained contraction the whole of the work may disappear. The work absorbed in a stretch must be used in driving some chemical reaction backwards, presumably the same reaction, but reversed, as that which produces work when a muscle is able to shorten.

For investigating these effects it is necessary to make simultaneous records of heat and work. Heat is recorded in the usual way with a rapid thermopile and galvanometer, the deflexion of the latter being amplified and displayed on one beam of a cathode-ray tube. A Levin-Wyman ergometer carries a tension lever, a lamp and a twin photo-tube, the potential changes caused by the movement of the shadow of the lever being amplified and recorded on the second beam. The ergometer is released by an electromagnet at any desired moment, the distance moved is adjusted by a screw stop, and the beginning and end of movement are recorded by electric contacts. The ergometer is driven by powerful springs, and its velocity is so nearly uniform that the work done can be taken as the average tension multiplied by the distance. The damping fluid of the ergometer is silicone (D.C. 200, 200 or 500 centistokes), the viscosity of which is less affected by temperature than that of oil.

In this way a stretch of any required extent and velocity can be applied during any desired interval of a contraction, heat, tension, and the beginning and end of stretch being simultaneously recorded on a uniform time base. The same method can be used for releases as for stretches.

Equipment for continuous collection of gastric juice in pouch dogs.

By B. Schofield and F. Watson. *The Buckston Browne Research Farm, and the Department of Physiology, Medical School, King's College, Newcastle upon Tyne*

Continuous collection of gastric juice has advantages:

(1) in the maintenance of the animal,

(2) in the investigation of secretion patterns of long duration.

For maintenance, continuous collection keeps the secretion from contact with the animals' skin and avoids erosions. The daily output can be determined, and this permits accurate replacement of water and salts. By this method it has been possible to keep in good condition animals with fully innervated pouches producing daily 300–500 ml. of juice high in acid and pepsin.

The pouches are fitted with a permanent silver cannula of a type designed by R. A. Gregory. It is of small bore and connects conveniently to a soft rubber tube. The dogs wear a short inner-jacket having holes for the fore-limbs and extending down the chest as far as the lower ribs. It fastens with large press-studs at the back, and has a draw-string at either end. A rubber football bladder clips with three press-studs to this jacket and hangs beneath the chest between the forelegs. The neck of the bladder, which points backwards, has a glass nozzle tied into it and connects with the tube from the cannula. Over the whole is put a protective outer jacket of the same general design, but larger and long enough to extend 2–3 in. caudal to the cannula site.

The bladders are used for day to day maintenance collection. In experiments where juice is to be collected over short periods, small polyvinyl chloride bags are used (see Fig. 1). They are triangular in shape and have two openings fitted with glass nozzles. They clip to the under jacket in place of the bladders. The opening at the apex points forwards and is closed with a glass stopper.

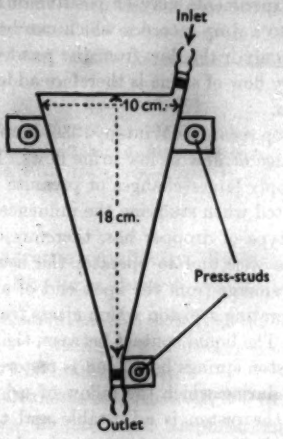


Fig. 1.

It serves to empty the bag. The other opening connects to the tube from the cannula. The bags have been made in several sizes, but the most useful has the dimensions shown and a capacity of about 100 ml. The material shows no deterioration in contact with pure gastric juice, but stomach contents will produce hardening.

The animals tolerate the clothing very well, and only occasional individuals worry the outer jacket. Strong linen material serves best for the purpose, and ex-government mattress covers form a satisfactory and cheap source of supply.

Dynamic ureter pressure urine flow characteristics. By F. R. Winton.
Pharmacology Department, University College, London

The analysis of urine pressure-flow curves, obtained by waiting for the value of the urine flow to reach a steady value at each ureter pressure, has revealed in the isolated kidney of the dog the phenomenon and magnitude of the intrarenal pressure (Winton, 1933, 1937). In anaesthetized dogs with innervated kidneys discontinuous changes in ureter pressure often produce

irreversible consequences, and such static pressure-flow curves are not easily reproducible. Factors such as diuretics which affect intrarenal pressure cannot, therefore, be assessed. To circumvent this difficulty the ureter pressure may be raised at uniform speeds ranging, in the present series of observations, up to 6 cm. water/min.

Urine flow in such experiments may be positive or negative. If the ureter cannula be connected to a drop recorder which can be raised, the pressure is determinate, however, only if the flow from the point of dropping is positive. A steady supplementary flow of saline is therefore added to the urine near the point of drop formation.

The usual type of drop recorder (Winton, 1936) involves drops too large for quick detection of change of flow at low urine flows. But smaller drops from smaller tubes would imply larger changes of pressure due to surface tension, which cannot be tolerated when studying the influence of changing the ureter pressure. A different type of dropper has, therefore, been devised in which each drop encounters a wire and so operates the usual electronic relay immediately it begins to emerge from the open end of a metal tube. The relay energizes a solenoid operating a piston which ejects from the end of the metal tube a 'drop' of liquid. The liquid contact between the wire and the tube thus vanishes so that the piston springs back, and is restored to its initial position within about 0·02 sec., during which the inflow of urine is obstructed by the piston. The stroke of the piston is adjustable and thus meters the liquid. 'Drops' of, for example, 5–10 cu.mm. can conveniently be obtained from metal tubes of 2–3 mm. diameter. This arrangement has certain advantages over orthodox droppers in addition to yielding smaller drops with smaller back pressure. Substantial changes in surface tension occur with change in concentration of urine, but they do not affect the volume of the 'drops' controlled by the stroke of the piston. Moreover, if a dropper be associated with an impulse counter such as Thorpe's recording device, there is inaccuracy due to uncertainty whether or no a drop has fallen unrecorded while the impulse counter returns to its base-line due to action from the time clock. This can be avoided by arranging for the time-clock circuit also to break the piston solenoid circuit, so delaying the discharge of the 'drop' until the impulse counter has completed its return to base.

Even so sensitive a drop-recording system cannot, however, signal reversal of urine flow quickly enough at the low flows encountered near the reversal point. For this purpose a small thermal flowmeter is inserted immediately beyond the ureter cannula. It consists of a 2 mm. diameter glass tube. Two platinum wires sealed through at opposite ends of a diameter have their points in the lumen about 1 mm. apart. The urine between them is heated by about 0·5 W. of 2000 cyc./sec. a.c. Either side of this hot spot lie a pair of single thermojunctions, and the difference in their temperature is recorded on

a galvanometer. Reversal of urine flow is signalled by reversal of galvanometer current, the sensitivity being highest at low flows, and the moment of reversal is signalled on the drum.

In recording on the smoked drum both the Thorpe impulse counter and the signal indicating reversal of urine flow are carried on a stage moving vertically and geared to rise or fall one tenth the distance moved by the urine dropper, the height of which determines the ureter pressure. The exact pressure corresponding to a given flow can thus be ascertained readily and accurately.

REFERENCES

Winton, F. R. (1933). *J. Physiol.* **78**, 9*P*.
Winton, F. R. (1936). *J. Physiol.* **87**, 20*P*.
Winton, F. R. (1937). *Physiol. Rev.* **17**, 408.

A galvanometer of extreme sensitivity. By B. C. Abbott, K. Copeland and A. C. Downing. *Biophysics Research Unit, University College, London*

The equipment demonstrated enables potential differences measured by a galvanometer similar to that described by Downing (1949) to be read to within 2×10^{-9} V. on a standard laboratory meter.

Movements of the galvanometer (sensitivity 4×10^{-9} A./mm., at 1 m. distance, internal resistance 25 ohms) are amplified up to 100 times. Light reflected from the galvanometer mirror illuminates a twin vacuum photocell. The resulting potential difference is amplified, and the output taken through a large series resistance to a microammeter. A small portion of the amplified output is injected into the primary galvanometer as negative feedback. This reduces the effect of variations in light intensity and of fluctuations in the photocell and amplifier circuit.

The primary galvanometer and amplifier unit is self-contained and portable and operates from batteries. No elaborate precautions have to be taken to avoid vibrations, since the slow microammeter integrates out rapid fluctuations of the sensitive galvanometer. The microammeter, which is easier to read than a light spot on a scale, can be placed at any distance from the rest of the equipment.

The average difference between successive readings of the zero at 10 sec. intervals, under ordinary laboratory conditions, corresponds to about 0·002 μV. This is equivalent to a temperature difference of 5×10^{-5} ° C. with a single thermocouple. Temperature fluctuations within the galvanometer box must be minimized and the control circuit be of copper throughout.

REFERENCE

Downing, A. C. (1949). *J. Physiol.* **109**, 10*P*.

Membrane potentials at the motor end-plate. By PAUL FATT and BERNHARD KATZ. *Biophysics Research Unit, University College, London*

With the internal micro-electrode (Graham & Gerard, 1946; Ling, 1948; Hodgkin & Nastuk, 1949) it is possible to obtain more direct measurements of membrane potentials than with the usual method of external recording. The new technique has been applied to the nerve-muscle junction, and 'end-plate potentials' have been recorded between inside and outside of individual muscle fibres.

Fig. 1. End-plate potentials recorded from the inside of muscle fibres. A. Curarized muscle fibre. End-plate potentials at five different distances (0, 1, 2, 3 and 4 mm.) from nerve-muscle junction. *S*, stimulus artefact. B. Normal muscle fibre (lower amplification and faster time scale). Action potential at the nerve-muscle junction (*a*), and 2·5 mm. away (*b*). Time scale: milliseconds.

The first part of the demonstration is made on a curarized frog's sartorius, stimulated through its nerve. The muscle does not respond mechanically, but an end-plate potential (e.p.p.) is set up at the nerve-muscle junction, and this is located by repeated insertions of a micro-electrode at different points of a muscle fibre (Fig. 1A). At the focal point, the e.p.p. reaches 20–30 mV. in amplitude, its size declining rapidly on either side of the junction.

In the normal, or decurarized, muscle, the e.p.p. rises at a much higher rate and leads to a propagating spike and contraction. The electric response at the end-plate (Fig. 1B, *a*) differs in a characteristic manner from the simple action potential recorded elsewhere (Fig. 1B, *b*). At the end-plate, the muscle spike (100–130 mV.) is invariably preceded by a discrete 'step' (30–55 mV., duration 0·5 msec. at 20° C.) and shows a marked 'hump' during the falling phase (90–100 mV. at 1·5–2 msec. after the start).

There is strong evidence that the end-plate potential is due to the depolarizing effect of acetylcholine (ACh.) which has been released from the motor nerve ending. A change in the size of the e.p.p. may be due to either of two causes: (i) a change in the amount of ACh. released from the motor nerve or (ii) a change in the ACh. sensitivity of the end-plate. The effect of

curarine in reducing the e.p.p. is known to be due to the second cause, viz. a reduced sensitivity of the end-plate.

The e.p.p. can also be reduced, and nerve-muscle transmission blocked, by lowering the external sodium concentration, but this appears to be due largely to factor (i), viz. a change in the amount of ACh. released. Over a wide range, the e.p.p. was found to be approximately proportional to the sodium concentration, while there were relatively small changes in the ACh. sensitivity of the end-plate. It appears, therefore, that there is some intimate relation between the amount of ACh. liberated from the nerve ending and the amount of sodium surrounding it. It is possible that $ACh.^+$ ions are released from the nerve terminal in exchange for Na^+ ions which enter it during its electric activity.

REFERENCES

Graham, J. & Gerard, R. W. (1946). *J. cell. comp. Physiol.* **28**, 99.
Hodgkin, A. L. & Nastuk, W. L. (1949). *J. Physiol.* **108**, 42*P*.
Ling, G. (1948). *Feder. Proc.* pt. 1, **7**, 72.

Apparatus for pharmacological assay. By J. L. Mongar and H. O. Schild. *Department of Pharmacology, University College, London*

The assay method is based on the principle of emptying the isolated organ bath completely before adding the test solution.

The apparatus consists of a small jacketed bath (volume about 2 c.c.) in which the tissue is suspended. A second jacketed vessel for pre-warming the solution empties into the bath. The assay is conducted on the '4-point' scheme and all operations are performed automatically. The advantages of the method are: (1) maximum sensitivity as the test solution is not diluted; (2) economy of test solution since the same solution can be used repeatedly; (3) absence of syringe injection error. Possible disadvantages are: (1) spontaneous contractions during the period of emptying; (2) contamination with preceding solutions. In practice we found the method to be accurate, sensitive, and rapid. For a histamine assay on the guinea-pig ileum a suitable period for a complete cycle of operations is 1 min. (this may be reduced to 40 sec.). With 12–20 cycles an accuracy of $\pm 5\%$ (P 0·05) is usually obtained. If the solutions are collected and used again, 3 c.c. of 5×10^{-9} histamine solution can be assayed with an accuracy of 10–15%.

Cannula for the simultaneous registration of the movements of the cervix and the corpus of the human uterus. By R. J. Fitzpatrick, W. C. W. Nixon and H. O. Schild. *Obstetric Unit, University College Hospital, and Pharmacology Department, University College, London*

A two-way cannula fitted with two small balloons is inserted into the pregnant human uterus in such a way that one of the balloons lies entirely within the cervical canal whilst the other lies well within the lumen of the corpus uteri. Contractions are transmitted to sensitive pressure recorders. Both recorders may show evidence of spontaneous contractions, whilst in other cases the muscle may be initially quiescent and activity induced by means of drugs. We found no obvious correlation between stage of pregnancy and occurrence of spontaneous contractions. Contractions of the corpus and cervix are not always synchronous, and in some cases the pressure changes recorded by the two balloons are opposite. Our results suggest that the human cervix possesses independent contractility.

The air-perfused bronchial tree. By Ourai Arunlakshana and H. O. Schild. *Department of Pharmacology, University College, London*

The isolated lungs of guinea-pigs are perfused with Tyrode solution through the pulmonary artery (for the injection of drugs) and with a stream of air through the trachea, air emerging from scarifications on the surface of the lungs. The air stream is controlled by a constant pressure head and resistance, and air pressure at the tracheal inflow is registered. The preparation lasts for many hours, gives a satisfactorily constant graded response, and is sensitive to 1–5 μg. histamine. It is suitable for accurate measurement of drug antagonism on the pA scale (Schild, 1947). pA values for various histamine antagonists determined in this way in bronchial muscle approximate to those previously found in intestinal muscle.

REFERENCE

Schild, H. O. (1947). *Brit. J. Pharmacol.* **2**, 189.

Staining properties of rods and cones in the retina of monkeys. By E. H. Leach and E. N. Willmer. *From the Laboratories of Physiology of Oxford and Cambridge*

The opportunity recently arose in Cambridge to make paraffin sections (4 μ. thick) of the retina of the red monkey (*Erythrocebus patas*). The eyes were removed under nembutal anaesthesia, the animal being killed in connexion

with another investigation in the laboratory. The tissues were fixed in Susa's fluid. Examination of sections cut both longitudinally and transversely to the rods and cones showed that the rods apparently fell into two distinct categories according to the staining qualities of their inner segments. In the majority the outer part of the inner segment stained darkly with azan and iron haematoxylin, while the inner part next to the external limiting membrane stained lightly. In the others, which became more numerous nearer the central area, the whole of the inner segment stained darkly. In cross-sections of the

Fig. 1. Macular region of the retina of *Macacca mulatta*, stained with Held's molybdic acid haematoxylin to show lightly and darkly staining cones. ×660.

inner segments the two types were clearly distinguished, the light type showing a ring-like structure with a central dark 'fibre', the dark type showing practically no internal structure.

Simultaneously, eyes of the macaque (*Macacca mulatta*) and the baboon (*Papio strepitus*) fixed under almost identical conditions with Susa's fluid in Oxford showed, when sectioned (11 μ.) in nitrocellulose and stained with Held's molybdic acid haematoxylin, Mallory's and other stains, a similar differentiation among the cones, especially those in the central area including those in the foveal centre itself. Both the central process running towards the bipolar cell and the outer segment also stained heavily. No continuous gradation between lightly and darkly stained cones could be observed (Fig. 1). At the junction of the inner and outer segments of these darkly staining cones, a refractile

spiral structure was seen arranged around the cone. The exact relationships of this structure have not yet been determined, but it may be connected with the pigment cells, rather than with the cones themselves.

Further independent examination of these two groups of sections showed clearly that both the cones and the rods could be subdivided into two visibly distinct groups. In these preparations about one cone in every four in the foveal centre has a darkly staining inner segment. This number at first rises and then decreases progressively towards the periphery. Among the rods, particularly those near the fovea, numerous individuals stand out clearly because of their darkly staining inner segments.

The dark staining of some of the cones of the foveal centre has already been commented upon by Kolmer (1936), but the method of preparation was not then specified. Similarly, the diagram in Fig. 66 of Wolff's *Anatomy of the Eye and Orbit* (1948), taken from Eisler, shows a clear distinction among the cones but not among the rods.

In certain retinae the staining of the cones is apparently reversed, though the underlying difference of morphology can still be recognized. In these preparations there is evidence of inferior fixation and probably this picture is the less reliable.

Whether or not these two visible forms of the rods and of the cones have any functional counterpart cannot be stated. The distribution in any given area is random and there is no evidence of clumping or of massing in special areas, except that, in general, dark-staining units are most numerous near the fovea. It is difficult to imagine that the random distribution can be in any way correlated with the effects of light or darkness on the form of the cells. It points to some more permanent distinction, though the actual picture obtained of each type may well change with function.

REFERENCES

Kolmer, W. (1936) v. Möllendorff's *Handb. d. mikroskopischen Anat. des Menschen*, 3, 295.
Wolff, E. (1948). *The Anatomy of the Eye and Orbit*. London: Lewis.

A method of recording heart-rate on the kymograph. By M. DE BURGH DALY and A. SCHWEITZER. *From the Department of Physiology, University College, London*

Several mechanical methods have been devised for recording heart-rate in animals. Most of them suffer from the disadvantage that either the recording is liable to become discontinuous when considerable changes in blood pressure take place (cf. Fleisch, 1930) or, with a mercury manometer, oscillations of its own may be set up at low frequencies. We have attempted to overcome these difficulties by employing a contact-breaker, each point of which is actuated by a separate rubber tambour.

The apparatus consists of two small rubber tambours, *A* and *B*, $\frac{1}{4}$ in. diameter, formed by a continuous sheet of rubber, *C*, clamped by six 8 B.A. screws between the brass pieces, *D* and *E*. Each tambour actuates a spring lever supported at one end on an ebonite block, *F*. A tungsten contact point is fixed to the other end of the lever. The contact points lie one above the other and the clearance between them can be adjusted by two 10 B.A. bolts, *G* and *H*. The tambours are connected to the blood-pressure cannula via two adjustable screw-down valves, *J* and *K*, and to the atmosphere by taps, *L*. The taps enable the air to be removed from the apparatus when filling it initially from the blood-pressure reservoir bottle.

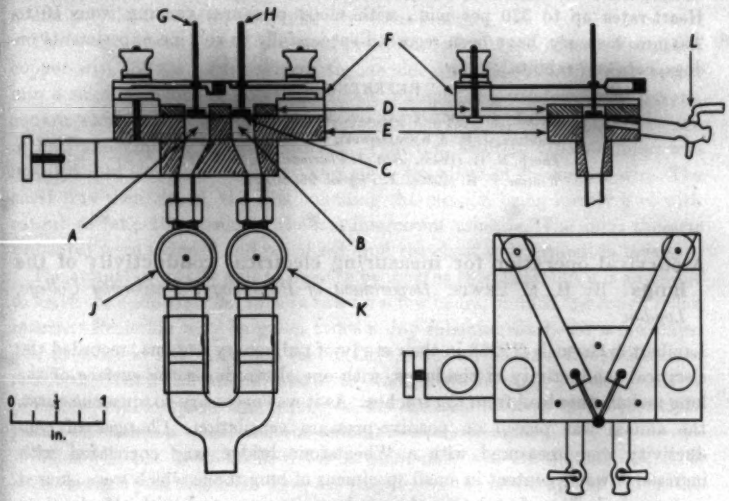


Fig. 1. Tambour unit.

In operation, with both valves *J* and *K* fully open, the oscillations of the two levers are equal and synchronous with the heart beat, so that the contact points do not move in relation to each other and therefore contact is not made. Valve *J* is then screwed down until the oscillations of the contact point actuated by the tambour *A* are almost completely damped. This results in the contact point supported by tambour *A* taking up a position somewhere between the limits of its former movement. Together with the other contact point, they now form a contact-breaker enabling a suitable electrical circuit to be broken with each cardiac systole. Valve *K* is only occasionally used when, with high pulse pressures, it may be necessary to damp to a variable degree the excursions

of tambour *B*. Otherwise tambour *B* may give a double oscillation with each heart beat corresponding to the anacrotic and dicrotic waves.

The advantage of this double tambour system is that any change in blood pressure causes the mean position of both contact points to move equally and in the same direction and therefore enables the heart-rate to be recorded irrespective of these changes.

Heart-rate can be conveniently recorded on the kymograph by using either a Thorp impulse counter (Thorp, 1948) or a Gaddum drop timer (Gaddum & Kwiatkowski, 1938) in conjunction with a ratchet counter (Winton, 1939). We found the latter method more convenient.

This method of recording heart-rate depends on an adequate pulse pressure. Heart-rates up to 320 per min., with blood pressures varying from 10 to 250 mm. mercury, have been recorded successfully in routine experiments on dogs, cats and rabbits.

REFERENCES

Fleisch, A. (1930). *Z. ges. exp. Med.* **72**, 384.
Gaddum, J. H. & Kwiatkowski, H. (1938). *J. Physiol.* **94**, 87.
Thorp, R. H. (1948). *Brit. J. Pharmacol.* **3**, 271.
Winton, F. R. (1939). *J. Physiol.* **95**, 60*P*.

A survival operation for measuring electrical conductivity of the lungs. By H. E. Lewis. *Department of Physiology, University College, London*

Lambert & Gremels (1926), in their study of pulmonary oedema, recorded the electrical conductivity of the lungs, with one electrode on the surface of the lung and another lead from the trachea. As it was necessary to open the chest, the animal was placed on positive-pressure ventilation. Changes of conductivity were measured with a Wheatstone bridge, and correlated with increase of water content in small specimens of lung tissue which were excised and weighed. They found that the conductivity increased with the development of oedema.

The present method employs this principle, but enables one to use the intact animal, breathing normally. Cats were anaesthetized with intraperitoneal veterinary nembutal (45 mg./kg.) and were given atropine subcutaneously (0·1 c.c. of 0·1% solution/kg.). An endotracheal tube was inserted under direct vision. It has been found preferable to use a flexible plastic tube of outer diameter 8 mm., inner 5 mm. If a rigid tube is used, complete alignment with the trachea must be preserved, since any kinking produces violent reflex spasm and coughing; this makes it almost impossible to control the lungs once the chest has been opened. The animal then received positive-pressure ventilation, and was placed on its side on a pad to produce a scoliosis of the thoracic

vertebrae. An incision was made along the line of an intercostal space, in the mid-thoracic region. Bleeding points were cauterized or tied before the pleura was opened. It was not necessary to resect a rib as the scoliosis and retraction of the ribs gave sufficient exposure.

Two small sterilized electrodes were clipped to the edge of a desired lung lobe, and the wire leads from them were brought through the paravertebral extensor muscles, their ends being taken anteriorly under the skin at about the interscapular region. It was necessary to have this length of 4–5 cm. of wire under the skin, for if it were brought to the surface immediately after passing through the muscles, the animal could pull it out. With this method there are no free ends exposed to the surface. The wires can be ensheathed in a thin polythene tube to minimize any tissue reaction.

The electrodes are small pieces of platinum foil 0·01 cm. thick. Varnished copper wire (38 s.w.g.) is soldered to one side and the electrode then sealed into a small envelope of polythene fabric, except for a window about 0·5 cm. square, which is to be applied to the surface of the lung, by bending the electrode in half and clipping it into position. Penicillin solution (10,000 units) was instilled into the thoracic cavity and along the track of the copper wire. The chest was then closed, the ribs bounding the incision being loosely tied with catgut to take the strain of the lost intercostal muscles. The intra-thoracic pressures were reduced and equalized, and the chest wall sutured in layers.

The animal is usually able to breathe by itself at this stage, but it is advisable to leave the endo-tracheal tube *in situ* for a few hours, or until the cough reflex returns. Penicillin may be given twice a day subcutaneously for a few days. The dressing is not disturbed for 10 days, after which time the stitches are removed. When the animal starts to walk there is some lordosis; this is probably due to spasm and injury to the extensor muscles of the back. It passes off within a week, and the animal recovers its agility.

When the cat is to be used for a pulmonary oedema experiment, it is anaesthetized; the electrode wires are palpated in the interscapular region, the skin is nicked, and the ends of the wires gently brought out. They may now be connected to the recording apparatus, and the electrical conductivity of the lungs can be measured with a 1000 cyc./sec. bridge without again opening the chest.

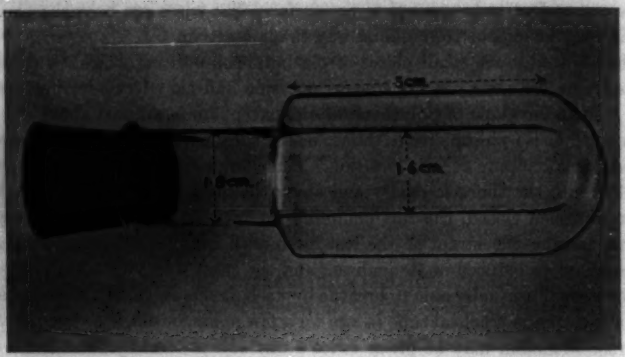
The expenses for this investigation were in part defrayed by a grant from the Medical Research Council.

REFERENCE

Lambert, R. K. & Gremels, H. (1926). *J. Physiol.* **61**, 98.

A new type of hexagonal roller tube for growing multiple tissue cultures. By CHARITY WAYMOUTH. *Chester Beatty Research Institute, Royal Cancer Hospital, London, S.W. 3*

The roller tube method for tissue culture, proposed in 1913 by Carrel (1913) and used by Löwenstadt (1925), Gey (1933), Lewis (1933), Coman (1942), Willmer (1942), Davidson & Waymouth (1943), White (1946) and others, has the advantage over the conventional hanging-drop and Carrel-flask techniques that large amounts of tissue can be easily maintained under comparable conditions in a single vessel. The chief disadvantages of most roller tubes



are the curvature of the walls and the poor optical quality of the glass, which make it impracticable to observe the living cells satisfactorily, and the difficulty of removing the tissue when it is desired to do so, for making stained preparations. Methods have been devised for removing cultures from the flasks or roller tubes in which they were grown, for sectioning, mounting and staining, but these are not always easy to apply without loss or damage to the cultures. Shaw, Kingsland & Brues (1940) described a roller bottle, made from a commercial 2 oz. bottle with a cover-glass cemented over a hole drilled in one wall. A modified roller bottle, based on the Carrel flask design, was described by Porter, Claude & Fullam (1945), in which the cultures were grown on two cover-slips inserted into the flasks and stuck on to the walls.

The type of tube shown in the figure is hexagonal in section (cf. Gey & Gey, 1936) and permits a number of tissue explants to be grown under uniform conditions on small cover-slips attached to the inner walls. The dimensions given are those found, after trials with larger and smaller tubes, to be most satisfactory and generally useful. Six $1\frac{1}{4} \times \frac{7}{16}$ in. cover-slips, each carrying two or three explants, can be placed in each tube. A small drop of plasma

clotted in the usual way (e.g. with embryo extract) holds each slip in place. A suitable volume of fluid medium for tubes of the size shown is 1·5 ml. The tubes are closed with sterile no. 7 rubber stoppers and mounted horizontally on a carrier which rotates at the required speed in an incubator.

The optical quality of the glass is such that low-power microscopic examination of the living cultures can be easily made. The glass is of such a thickness (1 mm.) and quality that, with thin (no. 1) cover-slips, examination of the cells with a $\frac{1}{6}$ in. ($\times 40$) objective is quite practicable. The cover-slips can be removed serially for fixation and staining, and the technique is particularly suitable for cytological study of cultures after exposure for six successive intervals of time to a single (e.g. drug-containing) medium.

I am grateful to Mr H. G. Flood and Mr J. A. Jenkins, of the Manufacturers Supply Co., Glebe Way, West Wickham, Kent (from whom the tubes can be obtained), for their helpful collaboration in tackling the problem of producing hexagonal tubing.

REFERENCES

Carrel, A. (1913). *Berl. klin. Wschr.* **50**, 1097.
Coman, D. R. (1942). *Cancer Res.* **2**, 618.
Davidson, J. N. & Waymouth, C. (1943). *Biochem. J.* **37**, 271.
Gey, G. O. (1933). *Amer. J. Cancer*, **17**, 752.
Gey, G. O. & Gey, M. K. (1936). *Amer. J. Cancer*, **27**, 45.
Lewis, W. H. (1933). *Contr. Embryol. Carneg. Instn*, **25** (no. 150), 163.
Löwenstadt, H. (1925). *Arch. exp. Zellforsch.* **1**, 251.
Porter, K. R., Claude, A. & Fullam, E. F. (1945). *J. exp. Med.* **81**, 233.
Shaw, D. T., Kingsland, L. C. & Brues, A. M. (1940). *Science*, **91**, 148.
White, P. R. (1946). *Growth*, **10**, 231.
Willmer, E. N. (1942). *J. exp. Biol.* **18**, 237.

Nervous control of the intra-ocular pressure.

By H. Davson.
Institute of Ophthalmology, University of London

In rabbits under light nembutal anaesthesia section of the cervical sympathetic frequently (but not invariably) causes a rapid rise in intra-ocular pressure on the same side, which may attain a value as high as 60 cm. H_2O; the pressure generally attains a maximum and then subsides, but an elevated pressure in comparison with the other eye may persist for hours. Stimulation of the peripheral end of the nerve causes a rapid fall in intra-ocular pressure on the same side, which is maintained for as long as the nerve is stimulated. Application of *N*-mustard to one eye causes a rapid rise in intra-ocular pressure in this eye; sometimes a rise occurs in the other eye; this contralateral response has been observed more frequently when the contralateral sympathetic was cut.

The acetylcholine content of the rat brain. By J. CROSSLAND. *Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*

When acetylcholine is extracted from animal tissues by grinding with 10% trichloracetic acid a maximal yield is obtained by extracting for about 2 hr. (Chang & Gaddum, 1933; Perry, 1949). A higher yield can be obtained from brain by freezing *in situ* with liquid air and extracting the finely ground solid, as described by Richter & Crossland (1949). The optimal time of extraction is then about 20 min. A comparison of the two methods gave mean values of 2·9 μg. acetylcholine/g. fresh brain by direct extraction and 3·3 μg./g. by extraction after freezing with liquid air, for a series of six anaesthetized adult rats. The difference was confirmed in further series of experiments.

Freezing with liquid air causes a disruption of the cells and apparently liberates acetylcholine. Even momentary thawing of the frozen brain before extraction with trichloracetic acid causes a serious loss of acetylcholine.

REFERENCES

Chang, H. C. & Gaddum, J. H. (1933). *J. Physiol.* **79**, 255.
Perry, W. L. M. (1949). *J. Physiol.* **110**, 20*P*.
Richter, D. & Crossland, J. (1949). *Amer. J. Physiol.* **159**, 247.

Selective absorption of amino-acids from the small intestine of the rat. By S. R. ELSDEN, Q. H. GIBSON and G. WISEMAN. *Departments of Bacteriology and Physiology, Sheffield*

Höber & Höber (1937) suggested that the rate of absorption of amino-acids was too great to be accounted for by simple diffusion, while Kratzer (1944) found that the rate of absorption of twenty-one different amino-acids was related to the molal volume. Using stereochemically specific methods of determination (bacterial decarboxylases, *Neurospora* and kidney oxidases) we have found that with several amino-acids, the naturally occurring compound is preferentially absorbed from a racemic mixture introduced into a loop of small intestine. In some cases (glutamate, histidine) the rate of absorption of the naturally occurring isomer was as much as six times as great as that of the unnatural isomer. We regard our findings as evidence in favour of the existence of a specific mechanism for the absorption of certain amino-acids.

REFERENCES

Höber, R. & Höber, J. (1937). *J. cell. comp. Physiol.* **10**, 401.
Kratzer, F. H. (1944). *J. biol. Chem.* **153**, 237.

The effect of electrical stimulation on the transport of radioactive phosphorus in the frog sciatic nerve. By F. GRANDE and D. RICHTER. *Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*

The sciatic nerves were freed by dissection as far proximally as the transverse processes of the vertebrae, in the decapitated and eviscerated frog. Oxygenated glucose-Ringer solution containing radioactive phosphate was passed at atmospheric pressure over the spinal cord through a needle inserted into the vertebral canal near the urostyle, to introduce ^{32}P into the spinal cord. After perfusion for 4–24 hr. ^{32}P was found to have entered the sciatic nerves. When one nerve was electrically stimulated, the rate of entry of ^{32}P was greater on the stimulated side.

The effect of stimulation in increasing the transport of phosphorus from the spinal cord into the sciatic nerve was confirmed in experiments in which the ^{32}P had been administered 2–5 days previously. Since the ^{32}P in these experiments was mainly organically combined and intracellular, the observations would agree with an increased flow of cytoplasm from the cell bodies into the nerve axons as a result of electrical stimulation..

Gastric secretion in response to insulin hypoglycaemia in Heidenhain pouches. By B. SCHOFIELD. *The Buckston Browne Research Farm, and the Department of Physiology, Medical School, Newcastle upon Tyne*

Heidenhain pouches in fasting dogs were washed out every 15 min. with warm water or N/200-HCl. The free and total acid of the washings were estimated. Steady base-lines were obtained and 1·5 units of insulin/kg. were then injected intravenously. In two out of four animals, a blood sugar of 50 mg./100 ml. or less resulted in a small but distinct acid secretion. In one of these the dorsal vagus trunk had been divided in the chest. This animal, but not the other, also responded to the sight and smell of food. The remaining two animals showed consistently negative responses.

Jemerin, Hollander & Weinstein (1943) found no secretory response to insulin hypoglycaemia in Heidenhain pouches. The above experiments, in which a more sensitive technique for the detection of secretion was employed, are consistent with the view that some of these pouches receive a small parasympathetic innervation.

REFERENCE

Jemerin, E. E., Hollander, F. & Weinstein, V. A. (1943). *Gastroenterology*, **1**, 500.

Ocular muscle activity evoked by stimulation of the frontal oculomotor cortex of the cat. By ROBERT B. LIVINGSTON. *N.R.C. Senior Fellow in Neurology* 1948–49, *Institut de Physiologie, Ecole de Médecine, Geneva, Switzerland*

In the anaesthetized cat it is possible to elicit conjugate deviation of the eyes by excitation of the medial surface of the frontal lobe (Hess, 1948; Livingston, 1949, 1950). After removal of the crystalline lens the horizontally acting ocular muscles can be sufficiently liberated to permit recording of their individual actions. The response to cortical stimulation consists of co-ordinate active shortening of the agonists and active lengthening of the antagonists, the right lateral and left medial recti, and the right medial and left lateral recti acting in concert. Both contraction and relaxation take place rhythmically, all four recti responding simultaneously with either slight shortening or slight lengthening. The rhythm is slow and does not seem to be related to the frequency of stimulation. The entire response consists of a series of these reciprocal changes in length which take place 'stepwise' and may demand as long as a full minute of continued cortical stimulation for completion.

Aided by a grant from The National Foundation for Infantile Paralysis.

REFERENCES

Hess, W. R. (1948). *Acta Helv. Physiol. Pharmacol.* **6**, 731.
Livingston, R. B. (1949). *J. Physiol. Path. gén.* **41**, 207 A.
Livingston, R. B. (1950). *Feder. Proc.* **9**.

The penetration of certain penicillins into the aqueous humour of the rabbit. By M. LANGHAM. *Ophthalmological Research Unit (Medical Research Council), Institute of Ophthalmology, London, W.C.* 1

In the rabbit the rates of penetration ($K_A \times 100$; Davson & Quilliam, 1940) into the aqueous humour, of penicillin G (sodium salt), procaine penicillin G (sodium salt), dihydro-penicillin F (ammonium salt) and penicillin K (ammonium salt) are $3{\cdot}80 \pm 0{\cdot}3$ (8), $4{\cdot}94 \pm 0{\cdot}6$ (7), $5{\cdot}80 \pm 1{\cdot}1$ (6), and $12{\cdot}45 \pm 1{\cdot}1$ (10) respectively; the percentage of the penicillin bound to plasma protein was found to be $50 \pm 1{\cdot}0$ (4), $27 \pm 4{\cdot}5$ (5), $75 \pm 2{\cdot}3$ (3) and $73 \pm 3{\cdot}2$ (4) respectively. The experimental results support the contention of Davson (1950) that the rate of penetration of nitrogen-containing compounds is increased with lowering of the degree of dissociation. It would appear that, of the compounds tested, penicillin K is the most effective antibiotic for treatment of intra-ocular infections.

REFERENCES

Davson, H. & Quilliam, J. P. (1940). *J. Physiol.* **98**, 141.
Davson, H. (1950). *J. Physiol.* **110**, 416.

PROCEEDINGS
OF THE
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A micro-titration attachment for the Stadie electrode system.

By B. Schofield and F. Watson. *The Buckston Browne Research Farm, and the Department of Physiology, Medical School, Newcastle upon Tyne*

The Stadie apparatus enables the pH to be determined by the glass electrode of small volumes of fluid protected from contact with the atmospheric air. The

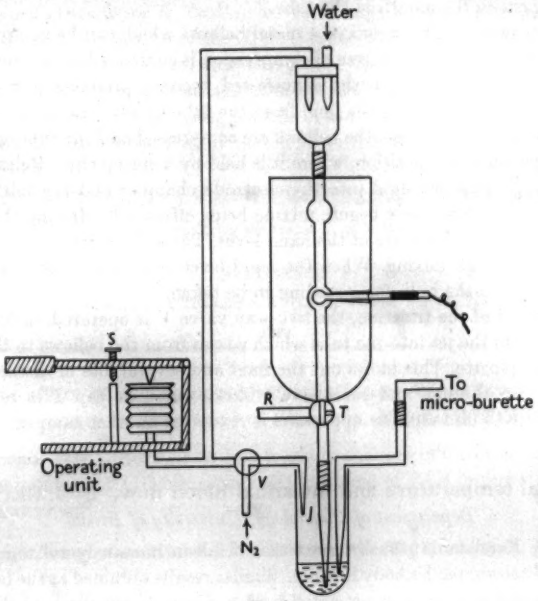

Fig. 1.

apparatus consists essentially of a pipette having two small bulbs each with a capacity of just under 0·2 ml. The lower bulb contains a small glass electrode. The bulbs are surrounded by a water-jacket. Connection to a calomel element

is made by saturated KCl solution in tube *R* (Fig. 1) and by a film of this solution in the tap *T*.

The attachments have been developed for the electrometric titration of the acid in the resting secretion of gastric pouches in dogs. Generally only small quantities are available, and a minimum volume of 0·5 ml. can be handled. This is titrated with 0·05 N-NaOH from a 2 ml. micro-burette.

A small glass chamber is connected to the upper end of the pipette by plastic tubing. Two small tubes enter it, one supplying distilled water for washing out, and the other providing the connection to the metal bellows in the operating unit. To the lower end of the pipette is attached an extension tip which reaches to the bottom of a small glass tube containing the sample for titration. The micro-burette also has an extension tip which is drawn to a fine point and descends to the level of the sample. To avoid contamination with atmospheric CO_2 during titration, nitrogen is blown over the solution from a small jet *J* which opens in the mouth of the tube.

The operating unit consists of a metal bellows which can be compressed by use of a hand-lever. The degree of compression is controlled by a screw *S*. This is set so that release from the compressed position produces just sufficient negative pressure to bring the fluid from the tube up into the bulb containing the glass electrode. In use, the bellows are compressed and the tube containing the sample placed in position, where it is held by a spring clip. Release of the hand-lever brings the fluid into the electrode chamber and the initial pH is read. The titration is now begun, mixing being effected by driving the fluid in and out of the bulb by use of the hand-lever. Three compressions will usually produce thorough mixing. When the hand-lever is released, the fluid always rises again into the bulb for a reading to be taken.

At the end of the titration, the two-way valve *V* is operated, diverting the nitrogen from the jet into the tube which passes from the bellows to the top of the Stadie pipette. This blows out the fluid and several lots of distilled water are now run through and successively blown out. The tap *T* is rotated to renew the KCl film and the apparatus is ready for another sample.

Rectal temperature and intestinal blood flow. By J. Grayson.
Department of Physiology, University of Bristol

Cooper & Kerslake (1949) observed that a fall in human rectal temperature frequently accompanies body heating. Similar results obtained at the beginning of the present work were investigated further, using electrically heated thermocouples (Gibb, 1933) at 39·5° C. With simultaneous recording from rectal thermocouples thus heated above blood temperature and from unheated rectal thermocouples it was found that exposure to cold usually produced a marked decrease in heated thermocouple temperature and a slight increase in unheated

thermocouple temperature; intravenous adrenaline or body warming had effects opposite to those of body cooling. These phenomena can only be interpreted as intestinal blood-flow responses, and as such resemble previous findings using colostomy subjects (Grayson, 1949). It is suggested that small fluctuations in rectal temperature might frequently have a similar circulatory basis.

REFERENCES

Cooper, K. E. & Kerslake, K. McK. (1949). *J. Physiol.* **108**, 40 *P*.
Gibb, F. A. (1933). *Proc. Soc. exp. Biol., N.Y.*, **31**, 141.
Grayson, J. (1949). *J. Physiol.* **109**, 53.

Blood groups in cats. By R. Holmes.* *Department of Physiology, University of Manchester*

Earlier work (Ottenburg & Thalhimer, 1915) suggests that cats possess no clearly defined blood groups of the *A*, *B*, *O* type. Preliminary cross-agglutinations among six cats indicated that two groups were present:

I. Those possessing only an agglutinogen in the cells.

II. Those having only an isoagglutinin in the serum.

Tests on 477 cats show that 97 % belong to the first group, and 3 % to the second group.

There are indications that there is a further group in the cat population.

* Work undertaken during the tenure of an M.R.C. Studentship.

REFERENCE

Ottenburg, R. & Thalhimer, W. (1915). *J. med. Res.* **28**, 213.

The effect of sciatic nerve stimulation on renal function. By S. Oleesky. *Department of Physiology, Manchester University*

Experiments were performed on cats to study changes in filtration rate (F.R.), *p*-aminohippurate (PAH) clearance and extraction of PAH during stimulation of the central end of the sciatic nerve.

The effect on F.R. *and* PAH *clearance during stimulation*

	Anaesthetized with pentobarbitone sodium	Decerebrate	Spinal
No fall	6	0	4
Slight fall	2	3	2
Marked fall	0	9	1
Total	8	12	7

In seven of the decerebrate cats the extraction of PAH was determined. Renal venous blood was obtained by placing a cannula in the left ovarian vein so that the tip lay in the renal vein. The mean extraction before stimulation was 75 % ± 3·2 (S.E. of mean of seven observations) and during stimulation was 76 % ± 2·9 (7).

Sciatic nerve stimulation in decerebrate cats reduced the renal plasma flow, without reducing the extraction of PAH. These experiments provide no evidence of a shunt during sciatic nerve stimulation.

Effect of strychnine on spinal reflexes in decapitated cats. By K. BRADLEY and W. SCHLAPP. *Department of Physiology, University of Manchester*

Records were made from the cut ventral root of L7 or S1 in response to peripheral nerve stimulation; D-tubocurarine chloride in amount sufficient to give muscular paralysis was used. Two-neurone and multi-neurone arc responses are increased by intravenous strychnine (0·05 mg./kg.). This is probably due to the recruitment of neurones from the subliminal fringe.

Primary inhibition was studied by applying facilitating and testing shocks to the nerve to gastrocnemius and inhibitory conditioning shocks to the anterior tibial nerve (Lloyd, 1946). Strychnine (0·05 mg./kg.) converts primary inhibition to facilitation.

REFERENCE

Lloyd, D. P. C. (1946). *J. Neurophysiol.* **9**, 419.

Effect of carbon dioxide on the spinal reflexes in decapitated cats. By K. BRADLEY, W. SCHLAPP and G. SPACCARELLI. *Department of Physiology, University of Manchester*

Records were made from the cut ventral root of L7 or S1 of submaximal two-neurone arc responses resulting from medial popliteal nerve stimulation. Carbon dioxide in the inspired air in amounts from 0·5 to 9 % produced only depression of the response. The effects were not due to anoxia or blood pressure changes. Brooks & Eccles (1947) made a similar observation with focal potentials. Lactic acid injected intravenously in amount sufficient to produce equivalent blood pH changes did not have this effect.

Carbon dioxide does not increase the inhibition resulting from conditioning shocks applied to the lateral popliteal nerve.

REFERENCE

Brooks, C. M. & Eccles, J. C. (1947). *J. Neurophysiol.* **10**, 349.

Effect of digitalis on the work capacity of the isolated heart. By A. Morgan Jones and W. Schlapp. *Departments of Cardiology and Physiology, University of Manchester*

Auricular fibrillation was induced in cat heart-lung preparations by a method previously described (Morgan Jones & Schlapp, 1945). The maximum work of which the preparation was capable without acute failure was tested at intervals by increasing the input to the left auricular cannula both with fibrillation and with normal rhythm. The capacity for work decreases steadily during an experiment but administration of digoxin interrupts or reverses the decrease. The extent of this digitalis effect is approximately the same whether fibrillation or normal rhythm is present.

REFERENCE

Morgan Jones, A. & Schlapp, W. (1945). *J. Physiol.* **104**, 23 *P*.

Observations on the action of the parathyroid hormone in the human subject. By M. D. Milne. *Department of Medicine, University of Manchester*

Administration of parathyroid hormone in normal human subjects did not result in any significant increase of urinary phosphate clearance; in a case of idiopathic hypoparathyroidism an abnormally low phosphate clearance was rapidly increased to normal levels after injection of small doses of the hormone.

An increased urinary excretion of phosphate precedes increase of urinary calcium. The simultaneous injection of solutions of calcium and phosphate ions into opposite arm veins produced similar results. This fact therefore does not necessarily imply that the hormone has any direct action on the renal excretion of the phosphate ion in the normal human subject.

The effects of pancreozymin on human subjects. By P. R. Duncan, A. A. Harper, H. T. Howat, S. Oleesky and H. Varley. *Department of Physiology, University of Manchester, Department of Physiology, Medical School, King's College, Newcastle upon Tyne, and Manchester Royal Infirmary*

On intravenous injection of pancreozymin in chloralosed cats there is an increase in the enzyme content of pancreatic juice and a contraction of the gall-bladder. The latter effect may be due to contamination of pancreozymin with cholecystokinin.

The duodenal contents have been collected from normal human subjects by continuous aspiration, and pancreozymin has been injected intravenously 30 min. after an injection of secretin. The lipase, amylase, trypsinogen and bile content of the duodenal fluid is increased after the injection of pancreozymin preparations. This confirms that the action of these preparations on man is the same as that on anaesthetized animals.